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PREDICT

Advancing Global Health Security at the Frontiers of Disease Emergence

DEDICATION

This book is dedicated to the PREDICT Consortium, a team that worked tirelessly for over a decade, often in some of the most difficult conditions on Earth to bring the project to life. The commitment and enthusiasm was inspiring and the fruit of their efforts is our collective success.

This book is also dedicated to those working on the frontlines of the COVID-19 pandemic and to the champions of One Health we have lost along the way, especially Dr. Tapan Dhole, Dr. Prime Mulembakani, and Dr. Douokoro Kalivogui "Kalis".

(Cover page) PHOTO: SIMON TOWNSLEY

PREDICT

Advancing Global Health Security at the Frontiers of Disease Emergence

"The only defense against the world is a thorough knowledge of it."

—John Locke

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This publication is the product of the PREDICT Consortium headquartered at the One Health Institute (OHI), School of Veterinary Medicine, University of California, Davis, USA.

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PREDICT-1 (2009-2014)

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USAID

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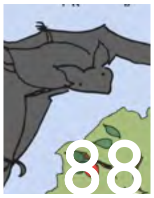

PART TWO COUNTRY CHAPTERS

PREFACE

One Health in Action Around the World

AFRICA & THE MIDDLE EAST

ASIA

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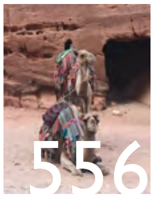
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PART ONE

"Viruses don't spread. People spread viruses.
We can change the way we behave on the Earth."
–Dennis Carroll

PHOTO: SIMON TOWNSLEY

EXECUTIVE

>6K

DEVELOPED the One Health Workforce by training more than 6,000 people in over 30 countries.

>60

STRENGTHENED laboratory systems and zoonotic disease detection capabilities in over 60 labs around the world.

>160K

OPERATIONALIZED One Health surveillance and sampled over 160K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

958 215

DETECTED over 1,100 unique viruses, including zoonotic diseases of public health concern such as Bombali ebolavirus, Zaire ebolavirus, Marburg virus, and MERS- and SARS-like coronaviruses.

SUMMARY

PHOTO: PREDICT VIET NAM

THE PANDEMIC ERA

From the first documented pandemic in 514 AD to COVID-19, infectious diseases, especially those with animal origins, have resulted in an enormous burden on humanity and have threatened our well-being, economic development, and global security. There are an estimated one billion cases of zoonotic diseases in people annually, and numerous other species are threatened or on the brink of collapse from a combination of anthropogenic pressures, including human-driven infectious disease transmission. Epidemics due to emerging viral diseases, in particular those caused by viruses with origins in wildlife hosts, are increasing in both frequency and severity. Spillover of these viruses from wildlife can be directly attributed to human activities. Human-induced landscape change, especially deforestation, intensification of agricultural production, and urbanization associated with human population growth, has disturbed ecological balances, altered transmission dynamics in all hosts, increased contact between people and animals, and driven viral spillover and amplification and spread of pathogens.

These trends coupled with our understanding that human, animal, plant, and environmental health are inextricably linked, have been a driving force for the use of a One Health approach for tackling the multifaceted challenges that emergence of infectious diseases represent. Together with USAID, the PREDICT Consortium pioneered a One Health approach to emerging virus and infectious disease identification, surveillance, and risk communication with collaborative investigations into risks associated with wild animal reservoirs, domesticated species in close contact with wildlife, and people at the highest risk of exposure to zoonotic viruses. Our objective was to use science and discovery to advance insight on features common to emerging pandemic threats globally and enhance risk reduction and preparedness locally, to improve capabilities for stopping pandemic emergence and spread at the earliest opportunity. From 2009-2020, the PREDICT project made unprecedented progress, strengthening capacity to detect viruses in high-risk settings for zoonotic spillover in many of the world's most vulnerable locations for infectious disease emergence and spread.

ONE HEALTH SURVEILLANCE FOR EMERGING VIRAL THREATS

The responsibility of having the opportunity to develop the world's most comprehensive One Health surveillance initiative to date was not taken lightly. Thus, we made a commitment to using a collaborative, cross-disciplinary, multi-pronged strategy consisting of concurrent human and animal surveillance, virus detection and discovery, and risk characterization paired with human behavioral and ecological investigations to explore virus sharing between animals and people and improve the world's understanding of the transmission dynamics that drive viral pathogen spillover, amplification, and spread. As a prerequisite, large-scale investments in surveillance capabilities and laboratory strengthening were made to help to establish or improve disease prevention, detection, and response systems in some of the world's most vulnerable locations for emerging infectious diseases. Our discoveries, insights, and lessons learned over the last 11 years have helped inform global research agendas, set new standards in zoonotic disease surveillance, enhanced laboratory capacity, expanded One Health training and education opportunities, and informed health policy and disease intervention strategies for achieving global health security.

VIRUS DISCOVERY & CHARACTERIZATION

The PREDICT project established a network of partnerships in 36 countries and strengthened the virus detection capacity of 67 collaborating labs. In these labs, we used consensus polymerase chain reaction (cPCR) as a tool for virus discovery, a technique ideal for detecting both known and new viruses in a large number of samples while remaining cost effective and feasibly implemented, even in resource-limited settings. Our in-country laboratory network performed 86% of all testing, an enormous feat considering >1.3 million tests were conducted. Together, we identified 1,173 unique viruses in wildlife, domestic animals, and humans. Of these, 958 were new viruses previously unknown to science, 215 were known viruses.

PIVOTAL DISCOVERIES

- A new ebolavirus, Bombali virus, in free-tailed bats in Sierra Leone and Guinea: this discovery marked the sixth identified ebolavirus to-date and is the first detection of an ebolavirus before disease has been reported in humans or animals.
- A new MERS-like coronavirus in an insectivorous bat from Uganda: as MERS is a significant zoonotic pathogen with high potential to cause severe disease in humans, we investigated the new virus' ability to infect human cells and determined that it does not likely pose a threat to human health. However, this discovery does highlight the role bats play as evolutionary hosts to a diverse range of coronaviruses, some of which are closely related to viruses that cause human disease.
- Ebola virus (EBOV) in a bat in Liberia >99% similar to the EBOV strain that caused the 2013-2016 West Africa Ebola epidemic: since the definitive animal host(s) of EBOV remains unknown, this discovery is an important step towards understanding the natural reservoir(s) of this deadly pathogen.
- Marburg virus (MARV) detected in fruit bats Sierra Leone >2,400 km from the nearest, previously known MARV-positive site: this discovery has brought to light the wide-ranging distribution of this deadly filovirus, allowing public health authorities in West Africa to include this virus within standard surveillance plans and enabling clinicians to add it to differential diagnosis lists to ensure rapid identification of the disease.

HIGH-RISK INTERFACES

The conditions where people live, learn, work, and play can all have an impact on one's health, well-being, and livelihood. An interface is a point of connection between different systems or organisms. The animal-human interface is the point where people and other animals (either domestic or wildlife) come into contact. Within the context of disease transmission risk, the interface is the epidemiologic point of contact that enables movement of a pathogen from one species to another, often into vulnerable human communities. The PREDICT Consortium collected samples from people, wildlife, and domestic animals at high-risk interfaces for disease emergence and investigated the social dimensions and human behaviors and practices that drive viral spillover and spread.

Learn more in the ***Special Feature: What is the Wildlife-Human Interface?***

EBOLA HOST PROJECT

In 2016, USAID and the PREDICT Consortium designed and implemented the Ebola Host Project (EHP) – the most comprehensive search for the animal origins of ebolaviruses and other closely related viruses since Ebola disease first emerged in Africa in 1976. EHP succeeded in revolutionizing our understanding of these viruses and changed the map of their distribution across Africa. Our efforts led to several landmark discoveries in bats, including: an entirely new species of ebolavirus (*Bombali ebolavirus*) in Sierra Leone and Guinea, *Zaire ebolavirus* (EBOV) in Liberia, and Marburg virus in Sierra Leone. These discoveries serve as frightening reminders that the cause of the 2014 West Africa Ebola epidemic and other lethal filoviruses continue to lurk at wildlife-human interfaces, with ongoing opportunities for re-emergence and potential for outbreaks with even higher tolls in human suffering.

Learn more in the ***Special Feature: The Ebola Host Project - Preemptive Detection of Emerging Disease Threats***

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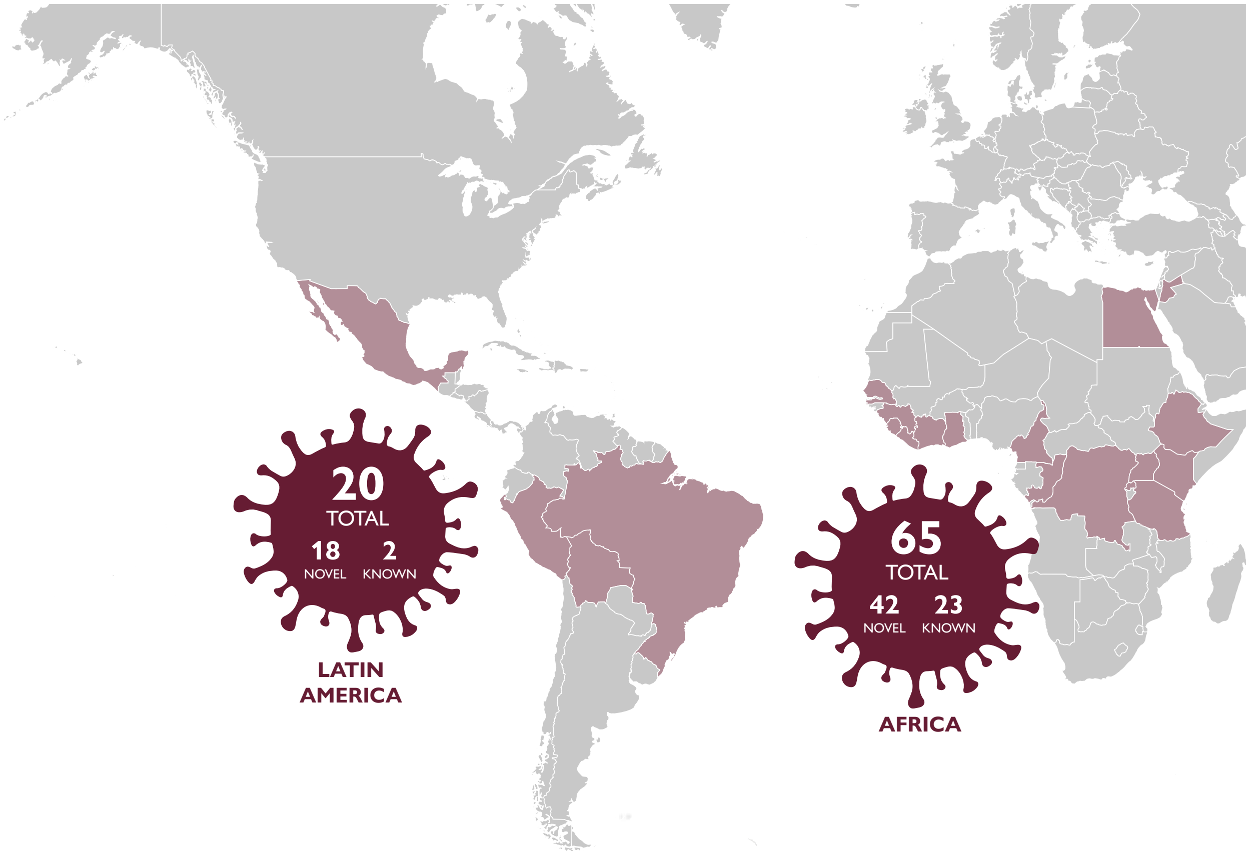
ADVANCING THE WORLD'S KNOWLEDGE OF CORONAVIRUSES

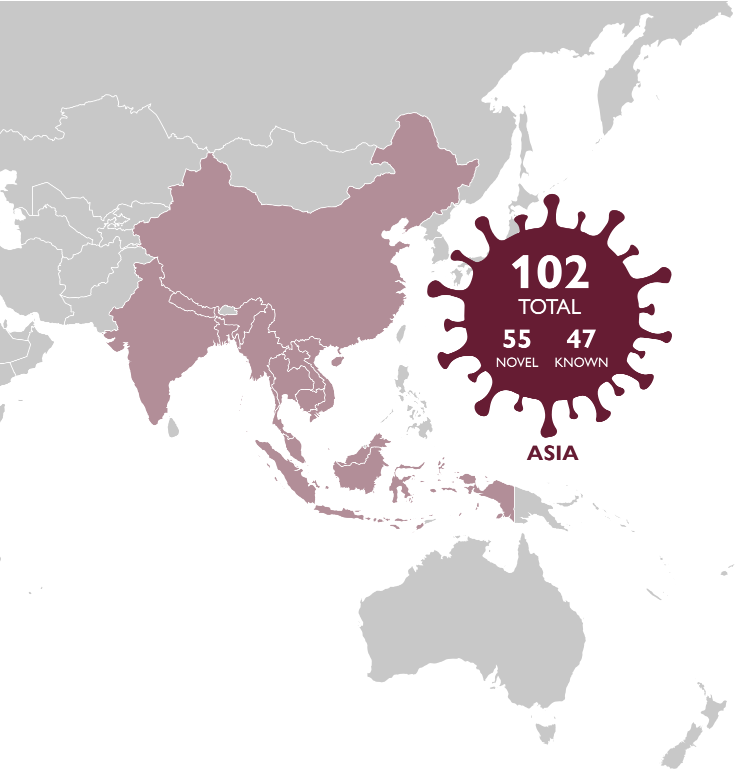
How does a virus related to ones that cause the common cold give rise to the most severe pandemic in the last 100 years? Recently emerged coronaviruses, such as SARS and MERS, are responsible for devastating epidemics, and COVID-19, which emerged in late 2019 unleashed a pandemic of biblical proportions. What do these viruses have in common, how do they emerge, what causes them to spread so rapidly following spillover? All three of these newly-discovered viruses are known or suspected to have evolved in a wild animal; are capable of infecting multiple, diverse hosts; and are suspected to have spilled over into people from animals, likely as a result of human behavior, often passing through an "intermediate" species that has close contact with humans.

Through our work and that of colleagues researching coronaviruses, we know that this group of viruses is well suited to spillover into new species and able to infect and even adapt to new hosts, characteristics common to pathogens that pose a heightened threat to health security. For over 10 years, PREDICT teams around the world worked to identify and characterize coronaviruses, searching for them in hotspots with intensive interfaces, such as live animal markets, caves where bat guano is harvested, and communities that border wildlife habitats. We advanced the world's knowledge of coronaviruses, detecting 177 coronaviruses in animals and people. Of these, 113 were coronaviruses previously unknown to science. As a result, the coronaviruses we detected have been used by other scientists to develop countermeasures, to test the potency of anti-viral drugs, and to help in the rapid assessment of the only antiviral in use for patients in the early days of the COVID-19 pandemic.



In early 2020, in response to the rapidly evolving novel coronavirus outbreak now known as COVID-19, PREDICT-trained scientists and our network of laboratories quickly mobilized, rapidly detecting and isolating the virus in their home countries before specific assays were broadly available. These leaders in One Health shared their knowledge and expertise to inform outbreak response strategies in the earliest stages of this pandemic, often enabling rapid action by public health systems.

Prior to COVID-19, our scientists provided guidance for policy makers and international institutions on reducing the risks of spillover from animals and spread among humans, working with the United Nations, World Organization for Animal Health (OIE), the International Union for Conservation of Nature (IUCN), and the World Bank. We also developed equitable approaches to reduce the epidemic and pandemic costs that coronaviruses and other emerging threats pose. These efforts include facilitating partnerships with particularly at-risk communities, those at the frontlines of spillover and emergence, working with high-risk individuals vulnerable to infection through their occupations and livelihood activities, and designing innovative strategies for reducing risks and changing human behavior for safer interactions with wildlife known as potential virus hosts.



Number of novel and known coronaviruses detected through PREDICT project surveillance (2009-2020) by region. Numbers across regions do not total as some viruses were detected in more than one region.

LIVING SAFELY WITH BATS

Given the importance of bats to ecosystems, as well as the preponderance of viruses with zoonotic potential that reside in them, we worked with partners in social science to develop a communications intervention strategy, centered on human-bat risk reduction and behavior change. Together with our One Health team, we investigated the most culturally aware, feasible, and effective intervention styles and selected a moderated picture book as the appropriate tool. Following the discovery of a new ebolavirus in bats in Sierra Leone, we worked closely with USAID and partners and developed the communications plan that incorporated these outreach materials, to ensure a well-coordinated effort with government and community stakeholders. Our resulting resource, *Living Safely with Bats*, is well-received tool that appears to be effective and appropriate for risk reduction for all bat-borne viral threats, especially those from filoviruses, such as Ebola, to coronaviruses, including SARS-related and MERS-related coronaviruses. This resource has been translated into 12 languages and put into practice at national and community levels in many countries in Africa, Asia, and beyond.

Learn more in the ***Special Feature: Living Safely with Bats***

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PHOTO: PREDICT INDONESIA

EXPLORING RISK AT THE FRONTIERS OF EMERGENCE

In caves in South and Southeast Asia, several species of bats roost and interact in enclosed spaces, enabling a microbial exchange that is otherwise not very common in natural settings. PREDICT and other scientists have detected multiple coronaviruses and documented ideal conditions for switching of hosts, that might be conducive to enabling human infection by these viruses. People visit these same caves, harvesting bat guano to sell or use as fertilizer for crops and gardens, putting themselves at risk of infection, perhaps unaware that their very livelihood centers around a high-risk interface for disease emergence.

A wide range of different viruses detected during PREDICT were found in animals that were hunted or sampled near people's homes and in and around their farms and fields. The viruses detected from four high-risk groups (coronaviruses, flaviviruses, paramyxoviruses, and influenza viruses) were more likely to be observed in animals sampled during the wet season, critical information for improving the probability of detection for cost effective surveillance efforts, as well as targeting of interventions. By using machine learning models of virus sharing among animal species, we helped identify viruses that could have a higher potential to infect people. Through these models, we found that coronaviruses have the ability to infect a greater number of species than viruses from any other group we investigated. Our data also show that bats, followed by rodents and shrews, are at higher risk for shedding viruses, especially coronaviruses, when compared to other wild mammals. Additional investments are needed to better understand the ecological and epidemiological features of bat-borne coronaviruses, especially the specific genetic features that could inform on their potential to infect people.

Our risk-based approach to One Health surveillance, focused on high-risk interfaces for disease emergence, allowing us to identify several animal-human interfaces with appropriate conditions for viral spillover, as well as control points for interventions. Live wild mammals in the animal supply chain, as well as live wild mammals sold at markets, had significantly more viruses than mammals sampled at other high-risk interfaces across the scope of PREDICT surveillance. Wildlife supply chains, including markets, represent a critical control point for needed interventions, as the conditions present at this particular interface include all of the necessary ingredients for virus evolution, amplification, and spread, the recipe for another pandemic.

Further, using serological techniques, we revealed previous pathogen exposure in at-risk populations, including those working in the wildlife value chain. Our data provide additional evidence for under-reported and unrecognized spillover events, events that are likely more common than we realize.

These findings shed light on where and how the next Disease X could spillover. Equipped with this knowledge we can better target surveillance on specific virus groups through cost effective efforts focused on at-risk human and animal populations. By using new technologies and tools, such as more rapid and sensitive molecular tests, safer and more humane approaches to sampling, additional investigations into the human behaviors driving emergence, and further advances in modeling and analytics, the PREDICT Consortium provided the data necessary to begin to identify viral threats and stop spillover in advance of the epidemics that threaten our very existence.

PHOTOS: PREDICT INDONESIA

FOREST TO TABLE

Wildlife supply chains are complex and diverse networks that involve the removal of wild animals from their natural habitats. These animals then enter a trade network spanning a mosaic of human-dominated landscapes, often from forests and sparsely populated rural areas to dense urban markets, sometimes across borders. We focused behavioral and biological surveillance efforts on a number of wildlife supply chains involving bats, rodents, and non-human primates intended for human consumption in Africa and Asia to gain insights into the role of the wildlife trade on the emergence of viruses of pandemic potential. We explored several points of contact between wildlife and humans along these supply chains to assess the risk of viral transmission at their respective animal-human interfaces. Our findings reinforce the need for wide scale policy and human behavior change to reverse the ever-increasing trend of wildlife-human contact fueled by habitat encroachment and the harvest and trade of wildlife species.

Learn more in our ***Special Feature: Forest to Table - The Wildlife Value Chain***

ALL SPECIAL FEATURES ARE AVAILABLE ON OUR WEBSITE

Scan with your iPhone camera or other code reader to access our web content

PHOTO: NIGEL WALKER

PREDICTING SPILLOVER

The PREDICT project's vision was bold and ambitious – to build capacity, gather data, and conduct surveillance in wildlife and people at critical high-risk interfaces so we could better prepare for and ultimately prevent pandemics. The goal was to disrupt the chain of disease transmission, snuffing out an outbreak before the first case amplified into a local epidemic and then spread internationally. But to stop spillover you have to know when, where, and how it will happen; you not only need diverse types of data but new approaches to analytics and modeling in order to forecast outbreaks.

We combined field data from our teams around the world and developed mathematical models to iteratively refine surveillance targets and improve the efficiency of virus discovery efforts. We worked collaboratively with our in-country partners to train and support their capacity for risk analyses and to make the tools, analytical products, and data outputs available to national decision makers.

KEY INSIGHTS:

- Global environmental change plays a major role in disease emergence.
- Zoonotic disease spillover risk is greatest in tropical regions with high levels of biodiversity, expanding human populations, and rapid ecological change.
- Four key factors underpin disease emergence: land use change, wildlife diversity, and livestock and human population density.
- Bats, rodents, and non-human primates were predicted to host a high number of zoonotic viruses, though we identified viruses in 196 species of mammals that were previously unknown virus reservoirs.
- Within China, southwest and southern China in particular are evolutionary hot spots for bat coronaviruses.
- We can effectively predict and map the risk of disease spread and identify epidemic control points, critical intelligence to rapidly contain and prevent another pandemic.
- It is also possible for a single evidence-based intervention to reduce spillover risk for multiple viruses.
- Pandemic mitigation measures, such as direct forest protection payments, could achieve a 40% reduction in risk, and pandemic prevention costs over a 10-year period would only equal ~2% of the global costs of the COVID-19 pandemic.

By working together with our network of global partners, utilizing data collected by our One Health teams around the world, and by pioneering new analytical strategies and open-source tools to analyze the risk of disease spillover, amplification, and spread, we pushed the field of virus forecasting forward. We envision an even brighter future, one in which One Health surveillance networks continue to expand globally with the support of national leadership and in which data from surveillance and discovery efforts are collected in near-real time, then shared with local modeling and analytical teams in public health systems to refine surveillance and to develop effective early warning systems for pathogen spillover.

ONE HEALTH OUTBREAK RESPONSE

Even before COVID-19, between 2010 and 2019, PREDICT teams provided critical support to governments and international organizations during 53 outbreaks in 16 countries, including outbreaks of Ebola virus disease and viral hemorrhagic fevers, animal and bird die-offs likely to have zoonotic origins, and avian influenza epidemics. PREDICT's outbreak response strategy incorporated the One Health approach at every step, as many outbreaks were driven by pathogen spillover from animals to people. At the request of governments, our teams provided critical assistance (diagnostic support, provision of supplies and personal protective equipment, sample collection assistance) and conducted disease investigations to help tackle outbreaks affecting human, domestic animal, and wildlife populations at their source.

In addition, we worked with our partners to strengthen outbreak response capabilities. We evaluated data and information sharing (in particular the reporting of Salzburg metrics for One Health surveillance), conducted outbreak simulation workshops, and developed a high-resolution mathematical modeling framework that simulates the spread of an outbreak and informs on high-risk areas for targeted and more effective control of epidemics.

These capabilities were put to the test in 2020, as our PREDICT-trained network jumped into action early in 26 countries, often representing some of the first responders working to first identify, then control, the newly emerging coronavirus as it suddenly spread across the globe. PREDICT partner labs detected some of the first cases of COVID-19 in their countries before a specific assay targeting the novel virus was available. These teams provided essential guidance and technical assistance to ministry partners and planners reacting to rapidly rising case numbers. This talented and proficient workforce joined emergency operations centers, led contact tracing efforts, developed risk communication strategies, and ultimately saved lives.

PREDICT OUTBREAK SUPPORT

PERU
2013: Leptospirosis & Dengue fever

BOLIVIA
2011: Hemorrhagic fever
2012: Yellow fever

SOUTH AMERICA

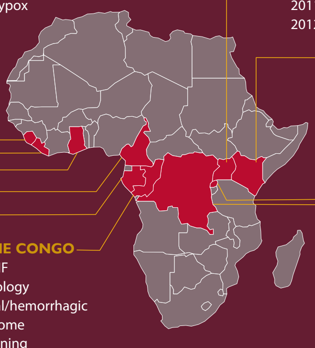
CHINA
2013: Influenza
2017: Swine acute diarrhea syndrome

MONGOLIA
2018: Bird die-off

BANGLADESH
2011: Nipah virus
Avian influenza
2017: Human Encephalitis
Crow Die-off, Dhaka
Crow Die-off, Rajshahi
2018: Human Encephalitis
Crow Die-off, Jessore
2019: Human Encephalitis

NEPAL
2017: Influenza H5N8

ASIA



LIBERIA
2017: Human diarrheal disease

SIERRA LEONE
2014: EVD
2017: Monkeypox

GHANA
2018: Lassa Fever

CAMEROON
2011: Yellow fever
2014: Chimpanzee Lesions
2016: Chimpanzee Monkeypox
2017: Bat die-off
Gorilla Mortality
2018: Unexplained hemorrhagic deaths

GABON
2012: Chikungunya

REPUBLIC OF THE CONGO
2010: Suspected VHF
2011: Unknown etiology
Neurological/hemorrhagic fever syndrome
2012: Alcohol poisoning

UGANDA
2010: Yellow fever
2011: EVD
2012: Sudan ebolavirus

KENYA
2017: Avian influenza
2017: Marburg virus disease

RWANDA
2017: Avian influenza

DR CONGO
2011: Suspected monkeypox
Suspected VHF & Lassa fever
2012: Chikungunya
EMCV
EVD
2013: EMCV
2014: EVD
2016: Animal die-off
Bonobo die-off
2017: Cattle die-off
EVD (May)
EVD (December)
VHF
2018: VHF, Bas Uele
VHF, Bikoro
EVD, Equateur Province
EVD, North Kivu Province

AFRICA

STRENGTHENING A WORKFORCE & SYSTEMS TO ADDRESS 21ST CENTURY HEALTH CHALLENGES

One of PREDICT's greatest legacies is the people and the systems our project worked to support and strengthen. Professionally implementing a One Health approach to disease surveillance requires a well-trained, technically skilled, multidisciplinary, and collaborative workforce. Surveillance teams need the knowledge and capabilities to safely and humanely collect samples from animals and people, conduct the laboratory tests that identify a virus and its corresponding health risks, and explore the social and behavioral factors associated with viral spillover and spread. We collaborated with communities and governments at the local, national, and regional levels and designed and delivered training in One Health skills for both the current and future workforce, essential competencies to prevent and respond to pandemic threats. We invested in the infrastructure required for successful surveillance and virus detection and tailored a curriculum and training program covering 19 One Health-related topics critical for project implementation and longer-term sustainability.

By leading the largest globally operational One Health project to date in terms of topical and geographic scope, we worked with partners to shift from largely conceptual interest in multisectoral collaboration to implementation and institutionalization of One Health. Our capacity building efforts spanned domains and silos and impacted many facets of existing health systems, including individuals, organizations, laboratory networks, governance structures, regulatory bodies, and policies. In just 11 years, we successfully trained, supported, and networked >6,000 wildlife, veterinary, and human health professionals from local, subnational, and national levels and facilitated knowledge-sharing and ongoing working relationships, breaking down historical barriers between sectors and disciplines.

TRAINING IMPACT

TOTAL TRAINED

>6,000

INDIVIDUALS

(19% STUDENTS**)**

♀ **41%** WOMEN

59% MEN ♂

20,341

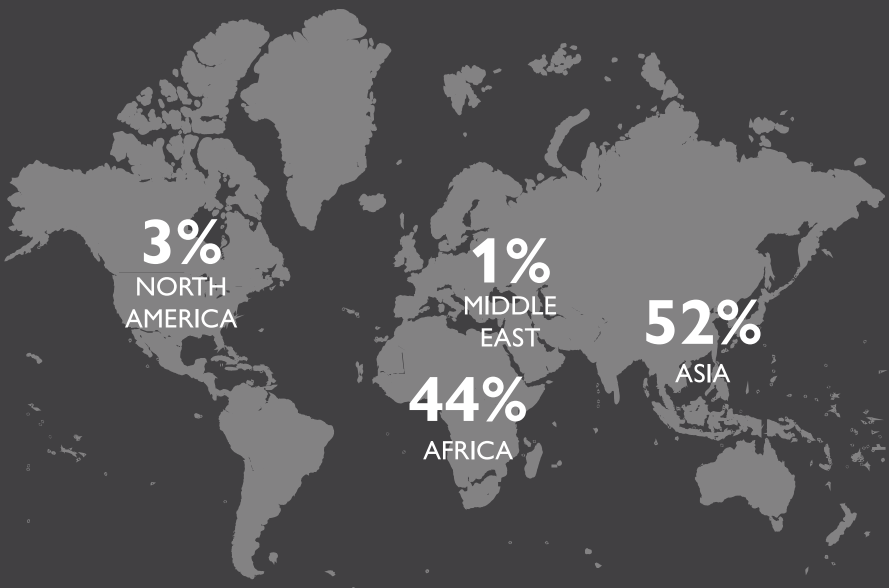
INDIVIDUAL TRAINING MODULES COMPLETED BETWEEN 2014-2020

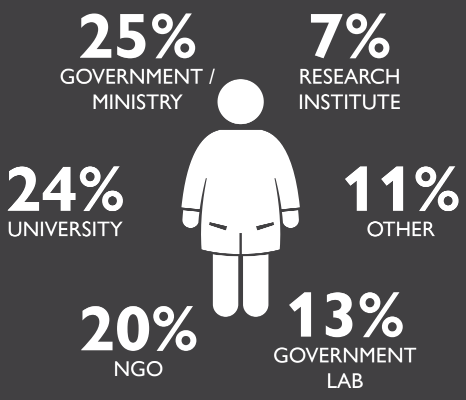
Scan with your iPhone or other code reader to access the PREDICT project's Standard Operating Procedures on our website.

Our work helped shape systematic changes that will make early warning and detection of disease threats from wildlife routine in national and subnational preparedness. We engaged and supported national mandates and global initiatives, seeking improvements in domestic financing and paving the way for new regulations, guiding frameworks, and policies. As a result, we are leaving behind more durable structures and systems, active and functional One Health platforms, and greatly strengthened multidisciplinary partnerships and networks.

In addition, after more than a decade of putting One Health in action, new leaders have emerged from our project, talented beneficiaries of years of intensive training, networking, and on-the-job experience at the frontlines of disease emergence. These leaders are taking the helm; integrating the One Health approach into global health security initiatives in their own institutions, countries, and regions; and continuing to put One Health in practice in their work.

Together, we have catalyzed positive change for development and instilled best practices for world class science and disease surveillance globally. In our pandemic era, the necessity for multisectoral involvement to achieve health security is increasingly obvious. New threats will continue to emerge from wildlife due to human behavior, causing disease and destruction. Meanwhile, wildlife health and disease surveillance remains massively underpowered, both in human resources and in financial investments, compared to domestic animal and public health surveillance systems. Despite the progress made by PREDICT, there are still major obstacles standing in the way of a safer and healthier future. We need further commitments and investments in One Health to continue approaches pioneered by this project, approaches that integrate disease surveillance in animal and human populations and that incorporate a holistic approach to capacity strengthening.





Training summary does not account for distribution of individuals trained early in the project in Latin America.

TOWARD A HEALTHIER FUTURE

Continued evolution of the One Health movement is fundamental to our forward progress as a society. Demand for animal-based protein and intensifying livestock production are still major drivers of shared diseases between animals and people, especially when combined with human population growth and landscape change that drive increased contact between people and animals. It is no wonder that zoonotic infections place an enormous burden on society with a disproportionate weight on the world's livestock keepers. However, "Disease X", the novel as-yet-undiscovered infectious disease that could threaten our very existence, will most likely have wildlife origins, and this risk is greater than ever.

The human footprint can be found now in every corner of the planet. Viruses in wild animals are therefore less likely to stay within the confines of their evolutionary hosts. Disturbance of natural ecosystems has altered disease dynamics and caused increasing movements and redistribution of wildlife with their ever-increasing need to accommodate and adapt to how humans are changing the world. Wild animals have declined with respect to every relevant aspect of their existence, in terms of abundance, numbers of species, genetic diversity, and sheer biomass.

Disease regulation is a process inherent to intact ecosystems. Declines in the quality of natural habitat due to deforestation, development, and conversion to cropland not only facilitate disease transmission by increasing movements of animals, but also simultaneously create more opportunities for animal-human interactions. Similarly, exploitation of wildlife through hunting and the wild animal trade creates close contact, crowded conditions, and movement of animals that further facilitate animal-to-human disease transmission. Wild animal species that are in decline because of habitat loss and exploitation, as well as those that are especially adaptable to human encroachment and even able to live in and around people's homes, have been shown to share more viruses with people. Transmission of a virus with pandemic potential is an extremely rare event, but if epidemiologic settings that select for a pandemic virus are increasingly common, we are all at heightened risk.

Over the past half century in particular, the natural balance has shifted. Industrialization, agricultural development, and globalization have improved human well-being and contributed to economic development, but the risks associated with ecosystem degradation and emerging infectious diseases are an unintended and as yet unmitigated outcome of our actions.

With development and advances in technology and associated changes to lifestyles, we seem to have started down a path on which we assume our health is independent of the health of the planet and the rest of its inhabitants, perhaps thinking that we are somehow insulated.

But the COVID-19 pandemic reminds us that our fate is not independent of nature. We need immediate action to preserve biodiversity, conserve and restore ecosystems, and investments in wildlife health to ensure sustainability of our own existence. This pandemic also reminds us that we are not alone. An animal-human interaction anywhere on the planet can affect us all if that interaction enables disease transmission of a pandemic threat. A virus transmitted among humans anywhere in the world can still move across borders, undetected in the earliest stages of a pandemic.

When we can travel around the world in less time than it takes for most viruses to cause disease symptoms in humans, the potential for the rapid escalation from a spillover event to a pandemic remains a global concern. Unless we reverse current trends over the course of this century, the rate that new microbial threats emerge will continue to accelerate due to growing socioeconomic and demographic factors, largely driven by the world's increasing population and interactions with animals. Success in preventing pandemics and the uncontrolled spread of epidemics requires thinking and acting differently. Revolutionary advances in health science and technology have made it possible to close the knowledge gap and discover what viral threats exist, but we must use these data and newly-developed tools to actively prepare for and prevent pandemics.

The information collected by the PREDICT Consortium should have lasting impacts on the world well beyond the timescale of the project. Our findings raised the flag for awareness of the rich virus diversity available for human infections, especially high-consequence pathogens, such as SARS-related coronaviruses, paramyxoviruses, and filoviruses, that should be considered alongside the previously-known influenza risks. We identified high-risk human behaviors at interfaces facilitating viral transmission, as well as the human-induced drivers for pathogen emergence. Through demonstrated products and data repositories, PREDICT has provided a vast resource of new, open access material for integration into future epidemiological investigations and pandemic prevention efforts.

The PREDICT Consortium has created innovative resources for policy makers to use in order to make scientifically-informed decisions when assessing the public health risk of novel viruses , including the PREDICT HealthMap site and SpillOver risk ranking web application. Our project provided the foundation and laid the groundwork for future development and better understanding of the risks posed by novel viruses of wildlife origin. We provided the structure and evidence base for globally-scaled efforts that are urgently needed to prevent and prepare for future pandemic events. If the COVID-19 pandemic has taught us anything, it is that we can't ignore the data that all point to increasing pandemic risk, and we must take informed action now to ensure global health security.

PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

Fortunately, visionary programs, such as PREDICT, shine a light on an alternate future. Our work was inherently collaborative and cross-disciplinary, building trust among colleagues around the world with the skills, expertise, and curiosity needed to continue investigations into emerging threats. One Health networks, such as the PREDICT Consortium, can quickly face down new threats. PREDICT's One Health team engaged partners in their vision and execution, from the highest levels of government to grass roots communities. One Health professionals engaged in transformative action, equipped with the conservation-minded participatory approaches established by the PREDICT Consortium, are now our pathway toward a healthier future. As we redefine our society and economies after the COVID-19 pandemic, we must support our next generation of leaders to combat emerging threats, conserve natural ecosystems and the environmental processes upon which we all depend, and to prioritize a sustainable and equitable future for all.

In the face of overwhelming uncertainty, we have the tools to prevent the next pandemic. Emerging infectious diseases are on the rise, but they do not have to define our near- or long-term future. Our collaborative research has dramatically increased our knowledge on the best approaches for detecting and discovering viruses of high pandemic potential that can move between animals and people. The PREDICT project provided many of the tools we need to help reduce the risk of deadly spillover events and ensure pandemics like COVID-19 never happen again – we just need to use them. We have developed and field-tested cutting-edge approaches to detecting viral spillover in high-risk populations and pioneered methods to mathematically model viral spillover risk – powerful examples of the tools needed for the fight to prevent future pandemics. Fortunately, those that have made the advancements above are today serving as the One Health-trained workforce (scientists and policy makers who explore the intersection of environmental, animal, and human health). They are prepared for and pushing back the frontlines of COVID-19 in many countries around the world. We are living in a world at risk of pandemic threats. If we heed the lessons learned from PREDICT and put them into onward action, allowing science to inform our health planning and response, we can look forward to a safer future for all.

(***OPPOSITE***) PHOTO: PREDICT MYANMAR

CHAPTER 1

One Health Surveillance for Emerging Viral Threats

PHOTO: CRISTIN WEEKLEY

KEY TAKEAWAYS

- From 2009-2020, the PREDICT Consortium pioneered a One Health approach to emerging infectious disease surveillance and risk communication at high risk wildlife-human interfaces.
- The world's most comprehensive One Health surveillance initiative to date, PREDICT used a multi-pronged strategy consisting of concurrent human and animal virus surveillance paired with human behavior investigations.
- Through this approach, we aimed to detect viruses, explore virus sharing between animals and people, and understand the transmission dynamics that drive spillover.
- By strengthening capacity, our partners in some of the world's most vulnerable locations for viral spillover and spread are now better prepared to prevent, detect, and respond to emerging health threats.
- Our discoveries, insights, and lessons learned over the last 11 years have helped inform global research agendas, set new standards in zoonotic disease surveillance, enhance laboratory capacity, present extensive training and education opportunities, and inform policy and intervention strategies for achieving health security.

At dusk on a humid night, as smoke from cook fires fills the air with the scent of roasting meat and spices, flashlights bounce off of a white-clad team of individuals working with gloved hands, faces behind respirators and face shields. They set up telescopic poles and stretch a long, mesh net around the eaves of a village church, and then wait. Soon they hear the chirps. Tiny shadows fly out from under the eaves and bounce into the net and tumble, tangled but unharmed. The white suits gently and quickly untangle the bats and place them into cloth bags, they hang the bags from a line, little patients safely waiting for their visit with the veterinarian. Nearby, a team member talks to villagers that have gathered around to watch the spectacle. She tells them about the PREDICT project and about One Health, she talks about zoonotic disease and living safely with bats.

THE PANDEMIC ERA

PHOTO: PREDICT DR CONGO

From the Plague of Justinian in 514 AD, the first documented pandemic, to COVID-19, infectious diseases, especially those contracted by humans from animals, have placed an enormous burden on humanity and have threatened our well-being and planetary health. There are an estimated one billion cases of zoonotic diseases in people annually (Grace et al., 2012) and numerous other species are threatened or on the brink of collapse from a combination of anthropogenic pressures and interspecies microbial exchange (Barnosky et al., 2011). Emerging viral diseases, in particular those of wildlife origin, are increasing in both frequency and severity (Tappero et al., 2015). Spillover of these viruses from wildlife can be directly attributed to human activities (Allen et al., 2017; Titcomb et al., 2019). Human-induced landscape change, especially deforestation, intensification of agricultural production, and urbanization, has disturbed ecological balances, altered disease dynamics in wildlife reservoir hosts, increased contact between people and animals, and driven viral spillover and amplification of pathogens (Allen et al., 2017; Titcomb et al., 2019).

These trends, coupled with our understanding that human, animal, plant, and environmental health are inextricably linked, have been a driving force for the use of a One Health approach for tackling the multifaceted challenges that human-induced emergence of infectious diseases represent. Together with USAID, the PREDICT Consortium pioneered a One Health approach to emerging infectious disease surveillance and risk communication and conducted collaborative investigations into wild animal reservoirs, interfaces with domesticated species in close contact with wildlife, and threats to people at high risk of exposure to zoonotic viruses. From 2009-2020, the PREDICT project made unparalleled progress strengthening capacity to detect viruses at high-risk settings for zoonotic spillover in many of the world's most vulnerable locations for infectious disease emergence and spread.

THE FIRST 5 YEARS (2009-2014)

In 2009, USAID launched the first phase of the Emerging Pandemic Threats (EPT-1) program to "strengthen capacities in developing countries to prevent, detect, and control infectious diseases in animals and people with an emphasis on early identification of, and response to, dangerous pathogens from animals before they can become significant threats to human health." (USAID, 2016). By design, EPT-1 put the One Health approach in action with investments in multiple projects across the continuum of domains linked to emerging infectious disease risk and vulnerability. The PREDICT project focused on detection and discovery of zoonotic diseases at the wildlife-human interface. From the start, the PREDICT Consortium recognized that to successfully detect zoonotic diseases, we needed to invest heavily in strengthening the infrastructure and core competencies required for safe and humane disease surveillance, essentially twinning a One Health capacity strengthening approach with our science and risk-based technical objectives (see *Chapter 7*). We used an evidence-based approach to target our investments and focus surveillance, relying on insights from maps and models that identified high-risk areas for emerging infectious diseases (see *Chapter 5*).

From 2009-2014, the PREDICT project worked with partners in 20 countries in some of the most vulnerable regions of the world for viral spillover and trained more than 2,500 individuals to safely and humanely collect and test samples from wildlife considered most likely to serve as hosts for emerging zoonotic pathogens. Samples were collected at high-risk, animal-human interfaces for disease transmission and then transported to host country partner laboratories for virus detection. Our conventional PCR-based virus detection strategy was designed to detect both known zoonotic viruses within virus groups that had caused disease and epidemics in the past, as well as novel viruses that might pose the next pandemic threat (see *Chapter 2*).

H1N1 Influenza
Pandemic begins
in USA
April
2009

2009
USAID initates Emerging Pandemic
Threats Program with the goal of
strengthening capacities in developing
countries to prevent, detect, and
control infectious diseases

MERS-CoV first discovered
in Saudi Arabia
June
2012

2013
Our China team investigated potential source
populations and the conditions for the genesis of the
2013 H7N9 virus outbreak using active surveillance,
screening of virus archives, and evolutionary analyses

This cost-effective detection platform was readily deployed to resource constrained laboratories in low- and middle-income countries, and when optimized, our partner labs screened samples for a wide range of viruses with high zoonotic potential. In just the first five years, the PREDICT project bolstered capacity in 32 labs and "detected 984 unique viruses in wild animals and humans: 815 novel viruses and 169 known viruses – the most comprehensive virus detection and discovery effort to date" (PREDICT Consortium, 2014).

PHOTO: PREDICT ETHIOPIA

USAID EPT-2 launches, PREDICT-2

2014

February 2014

Global Health Security Agenda launched

West Africa Ebola Outbreak declared

March 2014

August 2014

The PREDICT lab in Democratic Republic of the Congo assisted with diagnostics on a disease of unknown origin, leading to an early detection of Ebola virus, enabling a rapid response

In just five years, PREDICT-1 trained 2,500 One Health workers, sampled >56,000 non-human primates, rodents, bats, and other wildlife in 20 countries, improved laboratory capacity in 32 laboratories, and detected 984 unique viruses in wild animals and humans

September 2014

PREDICT-2: GLOBAL HEALTH SECURITY FROM THE EBOLA EPIDEMIC TO COVID-19 (2014-2020)

Before SARS-CoV-2 emerged in China, the West Africa Ebola virus epidemic from 2013 to 2016 showcased the devastating capability of a virus to disrupt civil society and cause massive health impacts. The epidemic, believed to have originated from a single spillover event involving an unknown wild animal and one human being from Guinea, quickly overwhelmed West African health systems and threatened to become a pandemic. Placed into historical context, this epidemic, with an estimated 28,646 cases and 11,323 deaths, was 90 times larger than the first recognized 1976 Ebola virus outbreak in Yambuku, Zaire. For the first time, Ebola would threaten global health security, infecting individuals from seven additional countries. Around the world, emergency outbreak response efforts were stretched to the limits of capacity, and the World Health Organization declared the epidemic a Public Health Emergency of International Concern in mid-2014. It took another two years, more than $3.6 billion in response funds, and the deaths of 513 health care workers before it was declared over in June 2016 (Centers for Disease Control and Prevention, 2019).

Further, the lack of trust in health systems, denial of the source of the problem, and burial practices involving contact with the deceased during the epidemic emphasized how critical understanding of biological, ecological, and social dimensions of zoonotic disease outbreaks is for effective disease prevention and control. This devastating epidemic revealed the urgent need for investments in disease

WHAT IS VIRAL SPILLOVER?

A viral spillover event happens when a virus moves from one species to infect another. Spillover can happen when a reservoir host comes in contact with a susceptible host population. For example, *Mastomys* mice are the known reservoir for Lassa virus in West Africa. Spillover of Lassa virus into communities is frequent, as the mice come into contact with people, their homes, and their crops.

2016

Zika virus outbreak in Americas

October 2016

Our team in China provides diagnostic assistance for an unknown disease affecting pigs, leading to discovery of swine acute diarrhea syndrome coronavirus (SADS-CoV), a virus related to HKU2 CoV found in bats

January 2017

One Health Economics Workshop, World Bank, Washington, DC

January 2018

“World Bank Operational Framework for Strengthening Human, Animal, and Environmental Public Health Systems at their Interface” is released

February 2018

Global Virome Project launched

March 2018

PREDICT responds to a request from the Government of Ghana to support the investigation of a suspected human Lassa fever case prevention, detection, and response capabilities across both animal and public health sectors and catalyzed further initiatives to identify host reservoir species (see *Special Feature: Ebola Host Project*), as well as to characterize spillover risks of emerging viral threats (see *Chapter 4*).

PHOTO: PREDICT GHANA

July 2018

Launch of the *Living Safely with Bats* risk reduction and behavior change communication resource, now available in 12 languages and adapted to regional contexts

August 2018

PREDICT's field and laboratory teams assist with investigation and response to a wild bird die-off event in Mongolia

May 2019

The US government approves the Global Health Security Stragety, outling their approach to strengthening global health security, including accelerating the capabilities of targeted countries to prevent, detect, and respond to infectious disease outbreaks

September 2019

PREDICT-2 primary award concludes

January 2020

PREDICT scientists in Thailand and Nepal assist with initial testing for SARS-CoV-2

March 2020

COVID-19 Pandemic declared by World Health Organization

March 2020

PREDICT receives six month extension to assist with COVID front line response and to explore other potential host species of SARS-CoV-2-related viruses

September 2020

PREDICT concludes

WHAT IS THE ANIMAL-HUMAN INTERFACE?

An interface is a point of connection between different systems. In the biological realm, an interface can be a point of contact between different organisms. The animal-human interface is the point where people and other animals (either domestic or wildlife) come into contact. Learn more in our *Special Feature: What is the Wildlife-Human Interface?*

WHAT IS SAFE & HUMANE SAMPLING?

Our PREDICT project teams were dedicated health and conservation professionals. All wild and domestic animals captured and sampled by our team were handled with the utmost respect and care, exceeding safety and international ethical standards for wild animal research. Samples, such as oral and rectal swabs and a small amount of blood, were collected from each animal for virus testing. After sampling, the animals were released alive, into their home habitat.

ONE HEALTH SURVEILLANCE

During the midst of the Ebola virus epidemic in 2014, USAID launched the EPT-2 program to help “countries detect viruses with pandemic potential, improve laboratory capacity to support surveillance, respond in an appropriate and timely manner, strengthen national and local response capacities, and educate at-risk populations on how to prevent exposure to these dangerous pathogens” (USAID, 2016). Under EPT-2, the PREDICT-2 project was refined and expanded, and our One Health strategy directly integrated social domains of disease emergence risk for a more unified approach to biological, ecological, and behavioral surveillance. PREDICT teams worked collaboratively to standardize approaches, protocols, standard operating procedures, and data collection tools to allow comparison of data and findings across countries and at scale. Then in 28 countries considered hot spots for emerging viral threats, our teams identified areas with high-risk wildlife-human interfaces and worked together with local professionals and community members to put One Health into action.

On the ground, the PREDICT project integrated local partners to form multidisciplinary One Health teams, consisting of behavioral and social scientists, veterinarians, public health and medical professionals, epidemiologists, ecologists, and data scientists. During the second five years, our One Health surveillance strategy was deliberately designed around the sampling of people and animals together (or concurrently) in the same space and time, across multiple seasons, and longitudinally over the course of three to four years. Through interviews with community members, we sought to explore the human behaviors associated with viral spillover and spread. With this multi-pronged strategy, we aimed to detect viruses, explore virus sharing between animals and people, and understand the transmission dynamics that drive spillover to better inform policy and intervention strategies for achieving health security.

SAFE & HUMANE SAMPLING AT HIGH-RISK WILDLIFE-HUMAN INTERFACES

Our locally-based One Health teams focused surveillance at high-risk interfaces within the epidemiological unit of potential viral transmission, otherwise known as a surveillance site. In each PREDICT-engaged country, our teams identified sites that best represented the complex ecology and social dimensions of emergence. In Cameroon for example, we explored the wildlife value chain and interviewed and sampled bushmeat hunters and traders from remote forests to urban markets (See ***Part 2: Cameroon***). In Nepal, our team engaged a community on the outskirts of Kathmandu that lived at the urban-rural frontier – farming, raising animals, and seamlessly integrating into the urban fabric of everyday life (see ***Part 2: Nepal***).

PHOTO: SIMON TOWNSLEY

In each participating country, our teams conducted surveillance at multiple sites, each site sometimes containing multiple wildlife-human interfaces. Our concurrent surveillance strategy was triangulated, as our teams collected samples from: 1) wildlife in and around dwellings, in nearby crops and natural habitat, and within and along the value chain; 2) livestock and domestic animals in close contact with wildlife and people (in partnership with FAO); and 3) people in close contact with wildlife and domesticated species. We enrolled patients with acute febrile illness of unknown origin in nearby clinics and hospitals and individuals from villages and communities considered high-risk for zoonotic disease spillover.

Our concurrent surveillance strategy (sampling of both people and animals) was implemented across Africa and Asia (Figure 1). We focused sampling efforts on people and wildlife, and we coordinated with the FAO to synchronize sampling of livestock and domestic animals at planned surveillance sites. In West Africa, we focused surveillance on wildlife in three countries heavily affected by the Ebola epidemic in an unprecedented effort to identify reservoir host species for the Ebola virus (*Special Feature: Ebola Host Project*). In Mongolia, home to a remarkable avian flyway between East Asia and the West, our team targeted wild birds to explore evolutionary and transmission dynamics of avian influenza.

In all PREDICT project-engaged countries, surveillance sites were characterized during each sampling event using a standardized observational survey designed to capture relevant ecological attributes and estimates of animal and human populations, and their interactions. Animal data were also collected through standardized forms that described animal condition and the type of contact animals had with people, described here as the animal-human interface. A detailed questionnaire, translated into 12 languages, was administered to all people we enrolled and contained questions on demographics, travel, hygiene, self-reported illness history, indirect and direct contact with domestic and wild animals, and knowledge, attitudes, and behaviors related to animals, animal consumption, and by-product use. In addition to the core questionnaire, we used 10 additional short surveys that we administered based on each individuals reported occupation or livelihood. Standardized forms for PREDICT data collection at the site, including at the animal-, human-, and specimen-level, were utilized by all teams throughout the project. Samples were collected following best practices in the ethical and humane sampling of animals and people, exceeding international standards in biosafety. All activities were conducted under protocols approved by the University of California, Davis Institutional Review Board and Institutional Animal Care and Use Committee, along with all relevant ethics committees and authorities in each country.

WHAT IS SYNDROMIC SURVEILLANCE?

Syndromic surveillance uses syndromes (groups of symptoms) in sick patients for rapid identification and enrollment of cases of from mystery diseases into disease investigations. We worked with health facilities (from large urban hospitals to smaller rural health centers) to enroll sick patients who came to the facilities with fevers or a recent febrile illness. With their consent, we collected samples and interviewed them about their symptoms and about their livelihoods, contact with animals, and other questions that might be associated with zoonotic disease transmission risk.

WHAT IS COMMUNITY BASED SURVEILLANCE?

We worked with local stakeholders and community members living or working near our animal sampling sites. After obtaining consent, we enrolled healthy individuals to take biological samples and conduct interviews, asking questions that explored zoonotic disease transmission risk.

A SPECIMEN'S LIFE HISTORY

1 CAPTURE

The safe & humane capture of an animal in the field

2 COLLECT

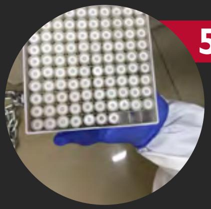
Biological samples are collected & catalogued before animal release

3 PRESERVE

In the field, samples are frozen & stored in a portable liquid nitrogen container

4 TRANSFER

When sampling is complete, specimens are transferred to permanent storage (freezers) in the lab



5 EXTRACT

Nucleic acid (RNA) is extracted from the samples

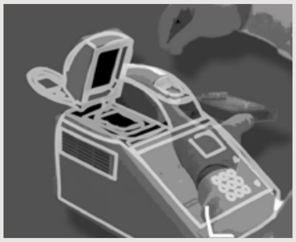
6 TEST

Using purified RNA, samples are tested for virus presence via consensus PCR (see *Chapter 2*)

VIRUS DETECTION & DISCOVERY

In 2014, we narrowed our virus detection and discovery strategy to focus on virus groups with the highest pandemic potential. RNA viruses, particularly viruses within the influenza virus, coronavirus, paramyxovirus, filovirus, and flavivirus groups, are of top concern among pandemic threats due to their potential disease severity and transmissibility characteristics. Known viruses within these groups are among the highest consequence viral pathogens anywhere in the world, and include ebolaviruses, Marburg virus, SARS-related viruses, Zika virus, yellow fever, dengue virus, West Nile virus, Nipah virus, Hendra virus, and highly pathogenic influenza viruses. Not coincidentally, these viruses are at the top of many countries' priority zoonotic disease lists. This testing strategy was designed with the One Health approach in mind, allowing us to use the same testing platform on specimens from both animals and people.

In over 60 labs, samples from animals and people were screened by the same consensus PCR platform developed in PREDICT-1, and presumptive positive test results were confirmed by genetic sequencing (see *Chapter 2*). We also used next generation virus sequencing to evaluate and optimize our virus detection and discovery platform. For wildlife hosts, we completed DNA barcoding to accurately identify the species sampled, especially if there was a virus finding from a difficult-to-identify animal. All test results were communicated to government partners within the Ministries of Health, Livestock, and Environment (or equivalent) in each country for approval in order for coordinated and sensitive release of all information to the public. Data produced by the PREDICT Consortium were made publicly available as soon as approval was received at **https://p2.predict.global/data** with the help of our partners at HealthMap.org.



HOW DO YOU DETECT A VIRUS?

Biological samples undergo 'extraction' to isolate the nucleic acid (RNA) present – this captures both the RNA of the host and any viral RNA present. Using a molecular method called polymerase chain reaction (PCR), a small RNA segment of interest is targeted and amplified – millions to billions of copies are created in just a few hours. This allows the targeted fragment to be 'sequenced,' a method that enables scientists to identify the organism from which it came, whether it be an animal or a virus.

SOCIAL DIMENSIONS OF VIRAL SPILLOVER & SPREAD

While the inclusion of the environment in One Health thinking has greatly increased our ability to tackle today's complex health problems (driven by climate change, human mobility, and land-use changes), there remain opportunities to better integrate "natural" and "social" systems in One Health approaches (Rock et al., 2009; Zinsstag et al., 2011; Woldehanna & Zimicki, 2015). From 2014 onwards, PREDICT's One Health approach prioritized integration of the social sciences to more fully identify and characterize behavioral risks associated with viral spillover and to generate targeted, evidence-based policy and intervention recommendations for risk reduction, disease prevention, and control. By integrating social scientists into One Health teams, we also helped encourage outreach and community dialogue that helped build the trust and relationships necessary for conducting our work and for collaboratively identifying and implementing solutions.

Human behaviors are complex, dynamic, and highly contextual. They are influenced by a myriad of socio-cultural factors that lie beyond the scope of traditional quantitative analytical methods. Along with behavioral factors that affect spillover risk, we also sought to explore and understand protective behavioral factors that could reduce risk from exposure to disease threats. Our behavioral risk strategy utilized a mixed methods approach to collect and triangulate quantitative and qualitative data at targeted surveillance sites. Quantitative data were gathered through PREDICT's standardized questionnaire. In 13 countries, qualitative data were also gathered through observational research, in-depth ethnographic interviews, and focus group discussions at selected high-risk interfaces.

WHAT IS COMMUNITY ENGAGEMENT?

The PREDICT Consortium included a constellation of partners around the world. In each country, we worked hard to build relationships and trust from the national to community levels. Our teams worked with the full range of stakeholders from project commencement to closure, orienting them about our objectives, working to understand their needs, and presenting our findings, insights, and recommendations.

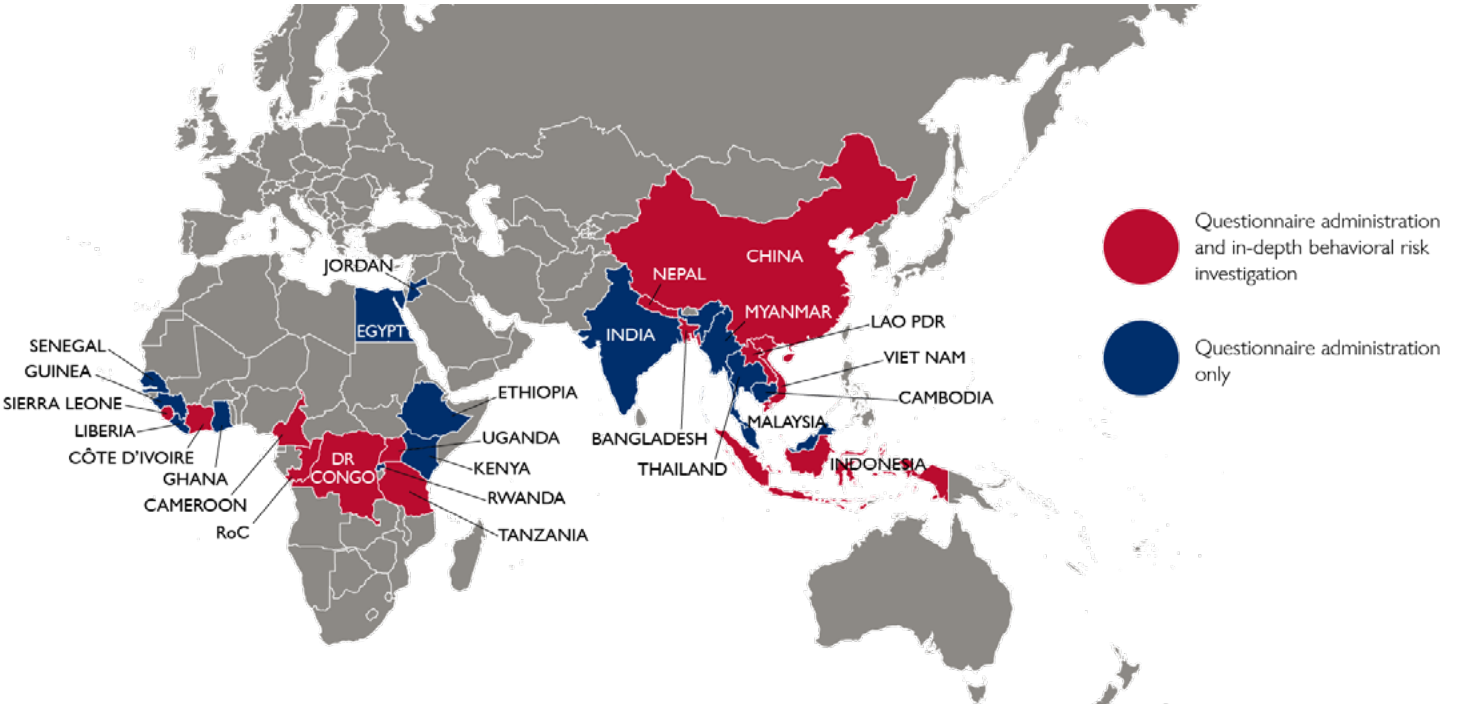


FIGURE 1. ***Global extent of PREDICT's behavioral risk investigations.***

Target populations for behavioral risk investigations varied across countries, and even across surveillance sites within countries, as we focused on the diversity of high-risk activities, occupational risks, and gender-specific hazards identified as most locally relevant or salient for zoonotic disease emergence and transmission risk. These interviews included people involved in the wildlife value chain (i.e. hunters, transporters, vendors, and consumers), butchers and abattoir workers, those in the guano fertilizer industry, and other individuals working with or in frequent contact with animals of interest. We enrolled individuals within a 10 km radius of animal sampling sites or at the local hospital or clinic (typically within 50 km of a wildlife sampling site) and conducted interviews using the standardized questionnaire. In the 13 participating countries, ethnographic interviews and focus groups were conducted with individuals in at-risk communities that our teams determined would yield the most insight. We completed 20,695 standardized questionnaires, more than 1,100 ethnographic interviews, and facilitated 104 focus groups engaging nearly another 1,000 individuals. Qualitative data were cleaned, coded, and analyzed. Along with established demographic analysis, we developed a uniform analytical framework to identify the exposures of interest, the direct and indirect wildlife contact modes of interest, and the variables associated with exposure and contact. Data were interpreted to gain insights and inform risk reduction and intervention recommendations that could be taken to scale (see ***Special Feature: What is the Wildlife-Human Interface*** and ***Special Feature: Living Safely with Bats***).

OUR JOURNEY

The findings, discoveries, insights, and lessons learned over the last 11 years have helped inform global research agendas, set new standards in zoonotic disease surveillance, enhanced laboratory capacity, and presented extensive training and education opportunities. In the chapters that follow, we have strived to chronicle PREDICT's journey, processes, discoveries, impacts, and recommendations in a comprehensive fashion that only begins to do justice to the dedicated efforts by our collaborative, multinational team who conducted world class science in some of the most challenging and remote corners of Earth.

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SPECIAL FEATURE

WHAT IS THE WILDLIFE-HUMAN INTERFACE?

PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

The animal-human interface is the point where people and animals (either domestic or wildlife) come into contact.

TO LEARN MORE ABOUT OUR GLOBAL INTERVENTION AT THE BAT-HUMAN INTERFACE SEE THIS

SPECIAL FEATURE: LIVING SAFELY WITH BATS

The conditions where people live, learn, work, and play can all have an impact on one's health, well-being, and livelihood. An interface is a point of connection between different systems or in biology, organisms. The animal-human interface is the point where people and other animals (either domestic or wildlife) come into contact. The animal-human, or wildlife-human interface can vary in intensity and frequency and represent a gradient of risk for viral spillover. Viral spillover risk is dependent on multiple factors, such as geography, climate, season, the presence or absence of wildlife hosts for potentially infectious viruses, and the type and frequency of human exposure to animals, their meat, or their excreta. Livelihood and occupation for example, can elevate exposure risk and therefore the risk of viral spillover. Just as a radiology technician is at higher risk for exposure to potentially dangerous radiation, a person harvesting bat guano from the floor of a cave is at higher risk for transmission of bat-borne viruses such as rabies or coronaviruses.

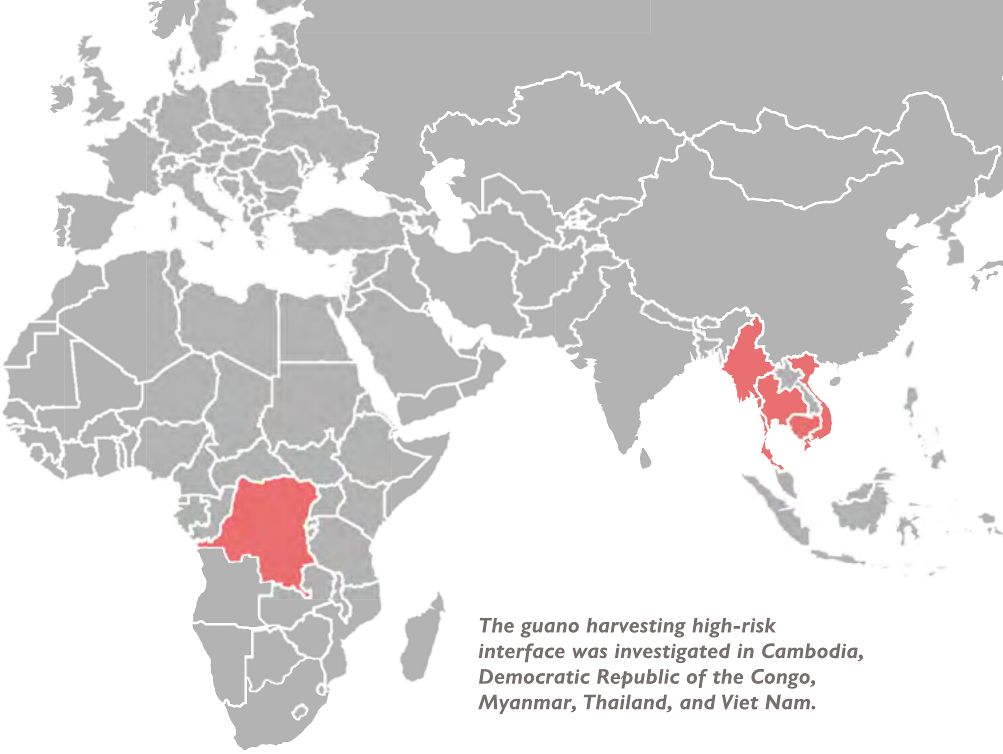
The PREDICT project collected samples from people, wildlife, and domestic animals at ***high-risk interfaces for viral spillover and spread***. Around the world, these interfaces varied according to local ecological, biological, and socio-cultural contexts. Here, our teams collected data and worked with local communities to better understand the multiple and interconnected forces driving risk of viral spillover and disease emergence. Our work enabled the development of locally relevant risk reduction and intervention strategies that can be taken to scale, regionally and globally wherever we identified clear patterns of risk or commonalities.

THE ANTHROPOCENE: NEW FRONTIERS FOR DISEASE EMERGENCE

In *Chapter 1*, we introduced the social dimensions of viral spillover at the animal-human interface. In this Anthropocene epoch, the human-influenced age, we are rapidly degrading forests, transforming landscapes, intensifying agricultural systems, and struggling to find balance between demands for economic growth and livelihoods and conservation for planetary health. As a result, we are creating new frontiers for virus emergence and new opportunities for long-hidden pathogens to emerge and spillover into people in novel and unpredictable ways. To better understand the human influence on disease emergence, our behavioral science teams aimed to explore the knowledge, attitudes, and practices that put people at risk for viral spillover.

Two forces driving contact at the wildlife-human interface are livelihood/economic need, and cultural or religious traditions. Here, we highlight cases from several particularly high-risk wildlife-human interfaces, using examples from PREDICT project countries where behavioral risk investigations using ethnographic interviews, focus groups, and structured quantitative surveys were embedded into One Health surveillance efforts.

RISKY BUSINESS: OCCUPATIONAL RISK FOR LIVELIHOOD & ECONOMIC GAIN



The guano harvesting high-risk interface was investigated in Cambodia, Democratic Republic of the Congo, Myanmar, Thailand, and Viet Nam.

'BLACK GOLD': GUANO HARVESTING IN SOUTHEAST ASIA

For generations, families in Hpa-An, Myanmar have been harvesting bat guano in sacred caves around their community. They use the guano to fertilize their crops and sell extra to supplement their income. Because these caves are sacred and also used as religious temples, footwear is removed upon entering and the guano harvesters rarely wear personal protective clothing or equipment such as gloves, masks, or eyewear. Across the border, in the Kang Meas District of Cambodia, farmers construct artificial bat roosts near their homes to collect guano, to fertilize crops and supplement family income. Here, the farmers also rarely wear protective gear beyond a simple hat or cloth gloves.

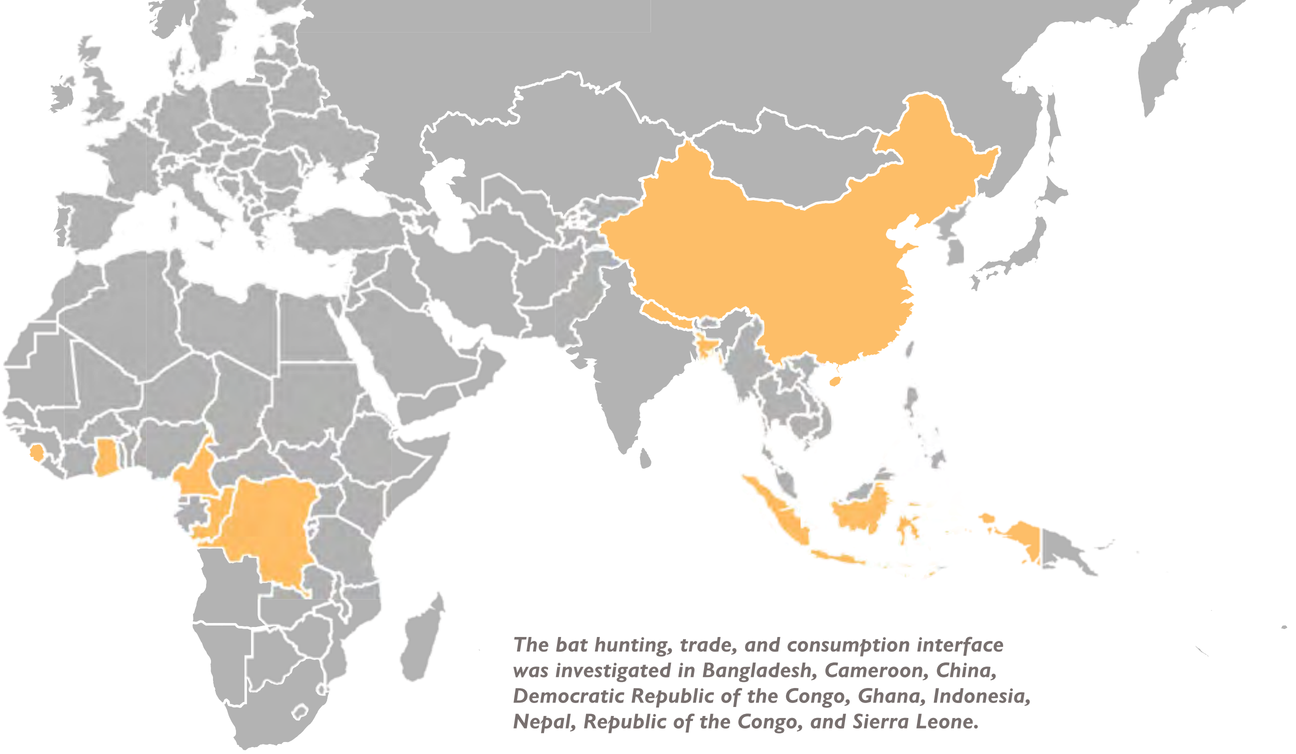
In these communities, people our teams interviewed reported minimal association between the handling of wildlife and increased risk of illness or disease transmission. In Cambodia, 72% of those interviewed were not aware of health risks from animal bites and rarely took steps to clean wounds when bitten. In Myanmar, guano harvesters often stay in the caves to take a break or eat lunch, unaware of infection risks despite reporting poor health following collection. In the Hpa-An caves, the PREDICT team discovered four novel coronaviruses and a paramyoxovirus in guano sampled from the cave floor, the same locations where harvesting, tourism and religious ceremonies are commonly practiced. In Cambodia, seventeen different viruses were found in bats of which 16 were newly detected. In both countries, our teams worked with all stakeholders to address risk at these interfaces and in these communities (see *Part 2: Cambodia* and *Part 2: Myanmar*).

HUNTING BATS: A MOST DANGEROUS GAME

In many countries around the world, bat hunting and consumption is a widespread practice as bats are an inexpensive food source, are often highly abundant, and are relatively easy to hunt during the daytime as they sleep in their roosts. Many bat species are also known as reservoir hosts for viruses known to cause severe disease in people (Rabies, Ebola, Marburg, SARS, and MERS), and hunting and consuming bats is an extremely high-risk practice with potentially serious consequences for human health.

In Cameroon, near the village of Sombo, lies a densely forested area with a large number of bats. Using guns and machetes, a small group of local hunters manage to capture up to 1,500 bats per month throughout the year. In the more urban area of Akonolinga, bats are hunted from trees and sold in local restaurants. Nearly 4,000 km away, in the Bombali district of Sierra Leone, a region hit especially hard during the West Africa Ebola epidemic, young men also hunt bats roosting in local mango trees, which they sell for food to earn money as supplemental income. These hunters sell the bats through a local distribution network, from individuals in the community to contract buyers seeking larger bulk orders. Because bat meat is less expensive than meat from domestic animals, people travel long distances to have the Bombali hunters help them hunt and obtain bats (Euren et al., 2020). Bat hunting, trade, and consumption is also common in Côte d'Ivoire, Democratic Republic of the Congo, and the Republic of Congo, and involves men, women and children, each playing role in the process and each with a different gradient of viral infection risk (see *Part 2 – Country Chapters*).

A common thread (both observed and reported in interviews) across each of these communities was a lack of protective clothing or gear when handling either living or dead bats. Both hunters and individuals preparing meat from the animals reported bat bites, scratches and knife cuts as common injuries. There was also a general lack of awareness or understanding of zoonotic diseases and how zoonotic pathogens could be transmitted from animals to people and cause illness. Even in cases where people acknowledged that viruses could spillover from wildlife into people, as documented during the Ebolavirus outbreak in the Democratic Republic of the Congo (see *Chapter 4*), the hunting, selling and consumption of bat meat continued.



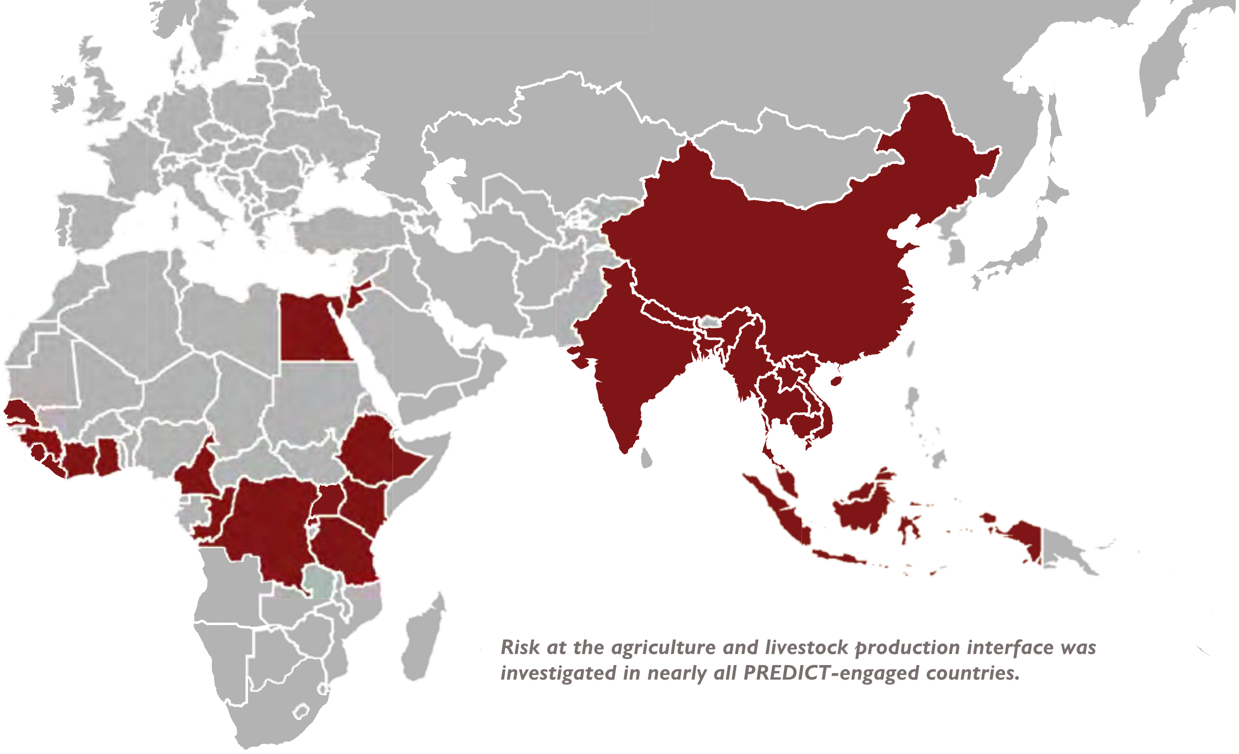
The bat hunting, trade, and consumption interface was investigated in Bangladesh, Cameroon, China, Democratic Republic of the Congo, Ghana, Indonesia, Nepal, Republic of the Congo, and Sierra Leone.

Changing behaviors and practices, even behaviors recognized or understood as a risk to health is extremely challenging, especially when those behaviors are influenced by economic need and long-held cultural practices. Simple solutions are often not easily adopted or put into practice, though slight shifts such as use of protective equipment during high-risk activities and regular use of soap to clean wounds and surfaces could have a significant impact in reducing infection risks.

RAISING RISK: FARMING & FORAGING AT THE ANIMAL-HUMAN INTERFACE

Viral spillover from wildlife is not unique to people, domestic animals are also susceptible to spillover and infection from newly emerging pathogens, sometimes with devastating consequences on animal health and livelihoods. Intensive livestock operations are particularly susceptible especially when they are near forests and highly biodiverse areas. Nipah virus for example, first emerged in 1999 from fruit bats in the tropical forests of Malaysia, infecting and causing mild illness in pigs, before spawning a devasting outbreak of deadly encephalitis in people.

In October 2016, a fatal swine disease outbreak was observed in a pig farm in Guangdong province, China, causing 90% mortality in newborn piglets. By May 2017, nearly 25,000 piglets had died across four farms. Preliminary testing suggested it was caused by a novel coronavirus. Working with multi-sectoral partners, the PREDICT team helped to quickly identify the pathogen, a newly detected coronavirus of bat origin named swine acute diarrhea syndrome coronavirus (SADS-CoV). The PREDICT team also conducted serological surveys with farm workers and sampled bats living around the farms to understand the transmission potential between bats and pigs and to help characterize the public health and behavioral risks of the new virus at this important wildlife-livestock interface.



Risk at the agriculture and livestock production interface was investigated in nearly all PREDICT-engaged countries.

Raising livestock is not the only high-risk activity associated with viral spillover. Agriculture also provides opportunities for wildlife contact and exposure to pathogens, through intensive cultivation where plantations take the place of old growth forest. In Bangladesh, for example, drinking raw date palm sap during the winter season is a cherished custom. The sap is harvested by shaving the bark from the upper trunk of date palm trees, which allows the sap to flow along the trunk into a clay collection pot overnight. Fruit bats, which also eat nectar, have learned to exploit this otherwise unavailable food by landing on the palm fronds and licking the sap as it flows, sometimes contaminating the sap with saliva and other excreta, such as when the bats urinate over the collecting pots. Many Nipah virus outbreaks in people have been associated with drinking of contaminated sap. For example, from 2001-2017, 289 people were infected in Bangladesh and 211 succumbed to the illness, a shocking 70% mortality rate. Nipah virus can be transmitted person to person, but not easily, and exposure to bats shedding the virus remains the primary driver of transmission. Because the wildlife hosts of Nipah virus, flying foxes of the genus *Pteropus*, have such a broad geographic distribution across one of the most populous regions on Earth and because the virus has such a high mortality rate in the absence of effective countermeasures (vaccines or therapeutics), there is concern that a more easily transmissible strain or a related virus may eventually spillover from bats into people that is more easily transmitted and could trigger a pandemic (see ***Part 2: Bangladesh***).

PHOTO: RICHARD NYBERG, USAID

FARM TO TABLE: THE RAT TRADE IN SOUTHEAST ASIA

In Viet Nam and Cambodia, the capture, trade and consumption of field rats throughout the Mekong Delta region is a common practice. Driving the trade are consumers in Viet Nam and Cambodia, some of whom report eating rats at least once per week because of their good flavor, low cost, and perception of rats as 'healthy, nutritious, natural, or disease free' (USAID PREVENT). PREDICT project teams in both countries conduced One Health surveillance and behavioral risk investigations at this unique wildlife-human interface, and documented a 34% prevalence of coronaviruses in rats sampled along this supply chain.

LEARN MORE IN OUR SPECIAL FEATURE: FOREST TO TABLE THE WILDLIFE VALUE CHAIN

RISK VS. REWARD: EXPLORING CULTURAL & RELIGIOUS TRADITIONS

CAVES AS SACRED TEMPLES

In both Cameroon and Myanmar, caves used for religious ceremonies also happen to be home to large colonies of bats. In the Akok Bekoe cave in Cameroon, villagers' weekly prayers and songs disturb the bats who fly in and out of the cave during the service. Water below the cave is the main source of drinking water for the community, creating a continual, potential risk of bat feces contaminating their water supply. In Myanmar, a system of caves outside the community of Hpa-An serves as both a sacred temple and a guano harvesting site, where footwear must be removed before entering whether the goal is to worship or collect bat feces. In the Hpa-An cave alone, four novel coronaviruses were discovered in guano samples. These behaviors have deep cultural and religious significance and our initial insights have illuminated the need for delicate and respectful risk communication, strategies that are culturally sensitive and that work with long-held traditional beliefs while shifting towards lower risk and protective behaviors.


PHOTO: PREDICT MYANMAR

EMBRACING CONFLICT: BALANCING TRADITIONAL BELIEFS, CONSERVATION, AND LIVELIHOODS IN GHANA

In Ghana, long-held cultural beliefs traditionally protected two species of monkey in the Boabeng-Fiema sanctuary - the black-and-white colobus (*Colobus vellerosus*) and the mona monkey (*Cercopithecus campbelli*). These monkeys co-existed peacefully with people in the villages of Boabeng and Fiema, which border the sanctuary. Historically, the monkeys were revered as "children of the gods" and protected from physical harm by local law (Densu, 2003). The villagers are mostly farmers, growing yam, maize, groundnuts and cassava as their main crops, and tobacco and cashew nut as a cash crop, though on a small, non-industrial scale.


PHOTO: PREDICT GHANA

However, human population growth, new religious sects arriving in the area, shifting attitudes, and encroachment on wildlife habitat have disrupted this balance and increased human-primate conflict and the subsequent risk of disease transmission between people and the monkeys. Monkeys commonly feed in the fields and orchards outside the sanctuary causing conflicts with farmers. Younger settlers sometimes disregard age-old taboos and local laws protecting the monkeys, hunting the animals that leave the safety of the sanctuary, and transforming wildlife habitat into fields and homes. In response, monkeys have become increasingly habituated and occasionally aggressive, raiding fields and even dwellings for food. Additionally, tourists visiting the sanctuary feed the monkeys, exacerbating their dependency on and habituation to people for sustenance (Appiah-Opoku, 2007).

Working with these local communities, the PREDICT project explored risks of viral spillover and spread at this unique human-monkey interface and partnered with the sanctuary and Government of Ghana to develop innovative behavior change and risk reduction strategies to discourage contact with and habituation of these traditionally cherished animals. Read more in *Part 2: Ghana*.

CLOSE TO HOME

We have coexisted for countless years, humans and bats, and some species of small insectivorous bats have adapted to roost very close to our villages and communities, often in our homes and other structures. In their nightly hunts, they hungrily consume a multitude of pests, insects that damage our crops, mosquitoes that carry deadly diseases such as malaria, Zika, and Dengue. But they are sometimes recognized as pests themselves as they contaminate our food and damage our buildings, causing stains on walls and floors from their urine and guano. A nuisance, but also a health threat as exposure to bats and their excreta bears a high-risk for viral spillover.

In the Bombali district of Sierra Leone, in the aftermath of the largest Ebola epidemic in history, massive outreach and communication campaigns on Ebola risks of infection led to a heightened awareness of potential health risks, especially from bats, a suspected virus reservoir. However, there was still a lack of knowledge and awareness about bats, including the benefits they provide to ecosystem services and other potential health risks they pose beyond Ebola. Through PREDICT's Ebola Host Project, our behavioral risk team found that people had regular indirect and direct contact with bats in and around their homes, that bats frequently impacted their sleep, there was a persistent smell of urine in homes, and it was even relatively common to be hit by bats flying in and out of roosts. Additionally, some people reported that bat feces sometimes contaminated their food or bath water, and food partially eaten by bats was occasionally consumed by children (Euren et al., 2020). Our Ebola Host Project team detected an entirely new species of Ebolavirus *(Bombali ebolavirus)* in samples taken from bats living in and around these same homes (Goldstein, et al., 2019), further evidence that bats close to home pose a significant though often underestimated health threat.

THE TIPPING POINT

These rich interfaces illuminate the complexity and urgency for understanding the connections between wildlife and the human behaviors that affect their health and wellbeing. As we head toward a tipping point, faced with climate change, mass extinction, and pandemics, we increasingly recognize that our behaviors, some long held and traditional, others just now illuminated through a new collective lens, are increasing our own species risk of infection. Identifying these social and economic influences, the behaviors and practices that drive human interactions with wild animals is an essential first step. Coupled with wildlife surveillance for emerging health threats, public health investments, and intervention strategies that balance conservation, development/poverty reduction and health goals, we can address these challenges, reduce risks, and find new solutions to our planet's crisis, One Health solutions.

> *By integrating the behavioral sciences to explore high-risk interfaces through a One Health surveillance strategy, the PREDICT project shed light on perhaps the most critical element driving viral spillover*: **ourselves**.

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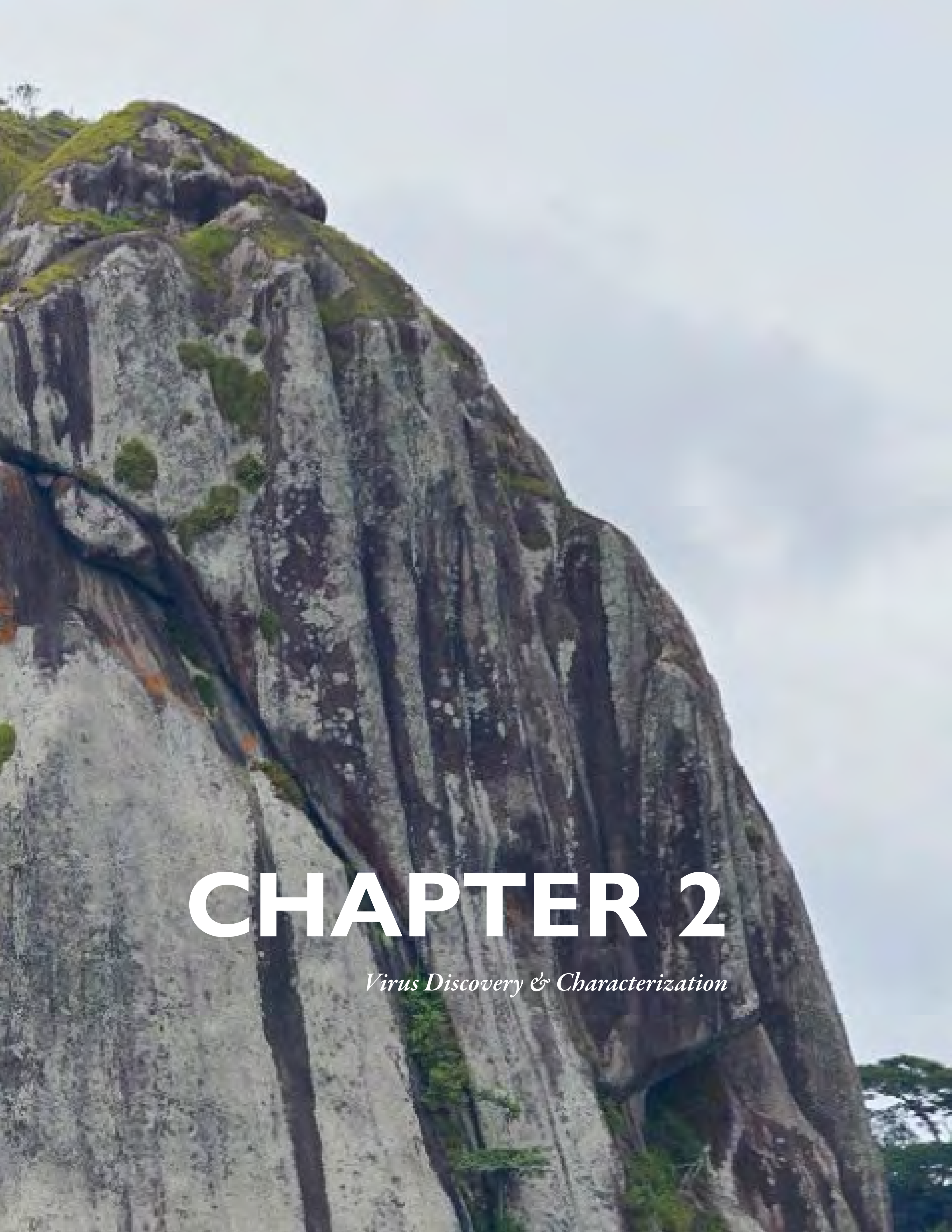
PHOTO: PREDICT CAMEROON



CHAPTER 2

Virus Discovery & Characterization

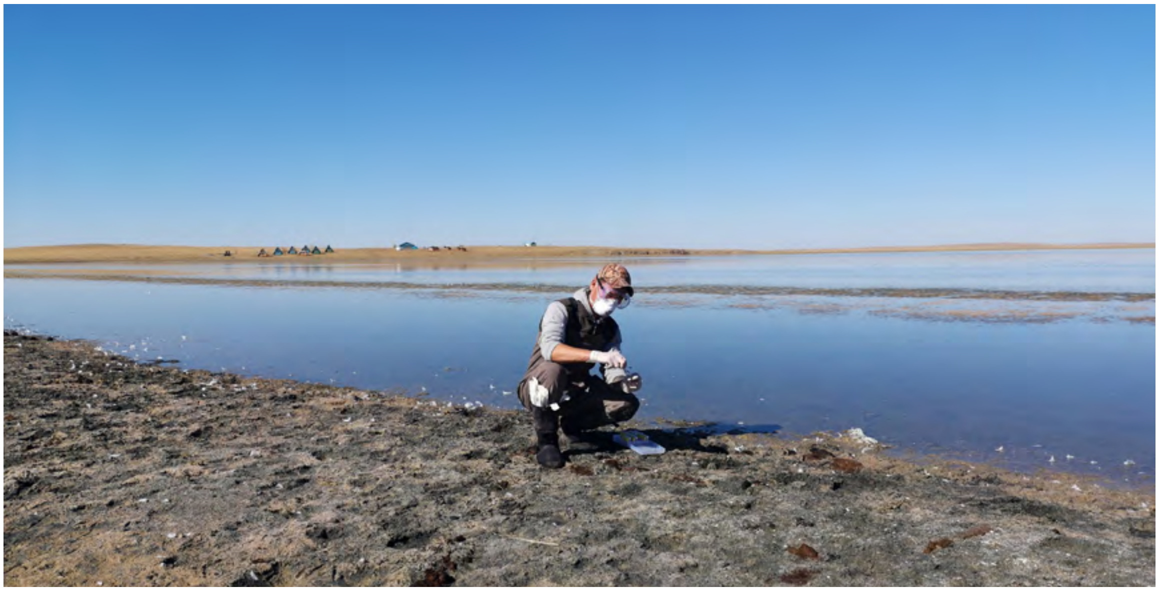
KEY TAKEAWAYS

- We detected 1,173 unique viruses in wildlife, domestic animals, and humans. Of these, 958 were new viruses previously unknown to science and 215 were known viruses.
- The PREDICT project built a network of partnerships in 36 countries and strengthened the testing capacity in more than 65 collaborating labs to detect viruses in animals and people. These labs outside the US conducted 86% of all PREDICT project laboratory testing.
- The PREDICT project implemented the use of consensus polymerase chain reaction (cPCR) as a tool for virus detection and discovery. This platform was needed for finding new viruses in different sample types from diverse species (>1.3 million tests were performed), as it is affordable and highly implementable, especially in resource-limited settings.
- Our virus findings were used to conduct epidemiological and ecological studies to evaluate the risk of viral spillover for potential pathogens. Results from these studies allowed us to engage local communities and educate them on the risks associated with close animal contact.
- We discovered a new ebolavirus, Bombali virus, in free-tailed bats within Sierra Leone and Guinea. This marks the sixth known ebolavirus and provides the strongest evidence to date that bats are the evolutionary hosts of ebolaviruses.
- A new MERS-like coronavirus was discovered in an insectivorous bat from Uganda through PREDICT project surveillance. As MERS is a significant zoonotic pathogen, we investigated the new virus' ability to infect human cells. While this virus does not appear to be able to infect human cells, we gained an understanding of the critical steps that lead to virus recombination. This discovery also highlights the role bats play as evolutionary hosts of potentially zoonotic coronaviruses.
- We found Ebola virus (EBOV) in a bat from Liberia that was most similar to the EBOV strain that caused the 2013-2016 West Africa Ebola epidemic. As the definitive host(s) of EBOV remains unknown, this discovery is an important step towards understanding the natural reservoir(s) of this deadly pathogen. We also discovered that distribution of filoviruses in nature is more widespread than previously recognized.
- We detected Marburg virus (MARV) in Sierra Leone, >2,400 km west of the previously known MARV-positive sites in Africa. Marburg virus can now be added to the list of public health threats, allowing for authorities in West Africa to include this pathogen during outbreak investigation and in surveillance plans. Our finding shows that Marburg virus is far more widely distributed than previously recognized.

On the first floor of a converted shipping container, a laboratory technician walks into a cramped room filled with freezers. Outside, a diesel generator kicks on – the back-up power supply keeping these freezers and the container's air-conditioning units running during the rolling blackouts that are common this time of year. From the freezer, the tech pulls out an aluminum rack filled with small cardboard boxes. She is searching for one in particular – it contains the next batch of samples in the pipeline for nucleic acid extraction and virus testing. In another room, as the deactivated samples thaw on ice inside a biosafety cabinet, she checks the lab computer to review results from a recent batch of testing. On the screen is a black background riddled with various soft, white horizontal bands. Three of the bands from this gel image look to be in the right place. Are these new viruses? Grabbing her phone, she sends the image to another lab tech in the US who agrees that they look interesting and that those samples should be prepared for genetic sequencing. This is virus discovery.

VIRUS DISCOVERY IN PANDEMIC PREPAREDNESS

Virus discovery efforts have an important role to play in controlling future epidemics. In the PREDICT project we took a broad approach to understanding viruses and virus risk in humans and animals by (1) **building global laboratory capacity** for virus discovery and characterization; (2) **using virus discovery to improve our understanding of zoonotic risk**; and (3) performing targeted surveillance to **understand spillover and emergence of specific, key pathogens**.



PREDICT Mongolia team member collecting samples at Sangiin Dalai Lake in response to reports of a wild bird die-off.

PHOTO: PREDICT MONGOLIA

OUR STRATEGY

We used a staged strategy of virus discovery and virus characterization to better understand the zoonotic potential of wildlife-origin viruses (Figure 1). In the first five years (2009-2014), we performed broad virus discovery of RNA and DNA viruses and tested samples across more than 20 virus groups. From 2014 onwards, we focused on the targeted discovery of viruses within five virus groups – coronaviruses, paramyxoviruses, filoviruses, flaviviruses, and influenza viruses. Each of these contain viruses of zoonotic concern and are considered high-risk for causing future outbreaks or pandemics.

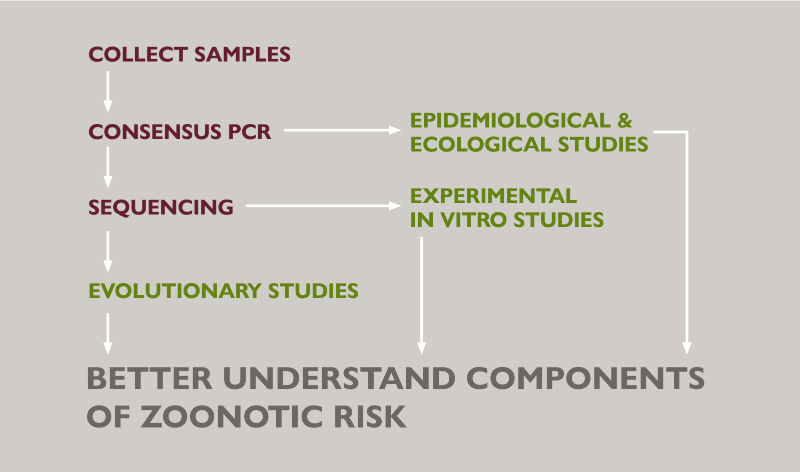
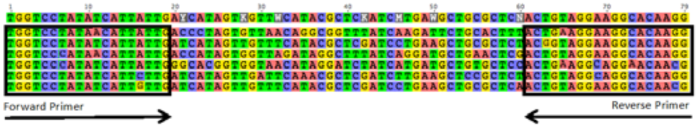


FIGURE 1. ***How the PREDICT project used virus discovery to understand zoonotic risk***

Consensus PCR

A PCR method that relies on the use of degenerate primers for the broad amplification of both known and unknown viruses



If more than one virus is present – both will be detected

WHAT IS PCR?

Polymerase chain reaction is a technique molecular labs use to amplify small segments of DNA for genetic analysis. This method creates millions to billions of copies of the targeted DNA strand, enabling it to be studied in detail.

Our main virus discovery tool was consensus polymerase chain reaction (cPCR) – a broadly reactive PCR method that uses molecular probes called 'degenerate primers' to bind and amplify conserved sequences shared by all viruses within a given virus group. By looking for parts of the genome that are common to all related viruses, cPCR can detect both known and unknown viruses. This technique is inexpensive, highly implementable in resource-limited settings, and is well-suited for finding new viruses in large numbers of samples.

> *From 2009-2020, the PREDICT project conducted over 1.3 million (1,363,103) cPCR tests on 223,080 samples from 115,391 animals and people. We discovered* ***958*** *new viruses and detected* ***215*** *known viruses – a total of* ***1,173*** *unique viruses. Within the five priority virus groups, we discovered* ***312*** *new viruses and detected* ***103*** *known viruses (Table 1).*

TABLE 1. ***Viruses in the five prioritized virus groups detected during the PREDICT project (2009-2020)***

	PREDICT-1 (2009-2014)		**PREDICT-2 (2014-2020)**		**LIFE OF PROJECT (2009-2020)**
VIRUS GROUPS	# KNOWN VIRUSES	# NEW VIRUSES	# ADDITIONAL KNOWN VIRUSES	# ADDITIONAL NEW VIRUSES	TOTAL
FILOVIRUSES	0	0	2	1	3
CORONAVIRUSES	36	73	28	40	177
PARAMYXOVIRUSES	13	79	14	113	219
INFLUENZA VIRUSES	1	0	1	0	2
INFLUENZA VIRUS SUBTYPES	1	1	10	0	12
FLAVIVIRUSES	1	5	7	1	14

> *Once we detected the genetic signature of a virus by cPCR, we used high-throughput sequencing (HTS) to sequence the rest of the genome.*

While cPCR is a powerful tool for virus discovery, it does have certain limitations. It only targets a small part of the virus genome, thus it provides limited information about a virus's evolutionary history or ability to infect people. Therefore, once we detected the genetic signature of a virus by cPCR, we then used high-throughput sequencing (HTS) to sequence the rest of the genome. HTS generates millions of genetic sequence fragments from everything in the sample, including DNA from the host (e.g. a bat or person), as well as any bacteria or viruses that are present. Computer algorithms then extract the virus genome fragments and stitch them together into complete genome sequences. From 2009-2020, the PREDICT project sequenced the complete genomes for 60 viruses, data that were used to investigate the potential zoonotic threats and risks posed by these viruses (see *How Can Virus Discovery Help Us Understand Zoonotic Risk?*).

MEET THE VIRUS GROUPS

Coronaviruses cause a wide spectrum of disease in people and animals. Some coronaviruses cause mild infections like the common cold, while others have been responsible for large-scale epidemics and pandemics, including SARS-CoV-1 (the virus responsible for SARS) and SARS-CoV-2 (the virus responsible for COVID-19). The PREDICT project has helped establish (1) that bats are a major evolutionary reservoir of coronaviruses, (2) that there are at least 3,700 different types of coronaviruses in bats, (3) that we can predict where different types of coronaviruses are likely to be circulating around the world, and (4) that evolutionary mechanisms like recombination have had a major impact on the emergence of coronaviruses in humans.

PREDICT detected 113 novel coronaviruses
and 64 known coronaviruses.

Filoviruses can cause severe hemorrhagic disease in humans, with case fatality rates reaching 50-90%. Ebola virus (EBOV) is a well-known example of a filovirus. In 2013-2016, EBOV was responsible for a very large outbreak in West Africa; more than 28,000 people were infected and more than 11,300 people died. The PREDICT project has contributed significantly to our understanding of the origins and geographic distribution of filoviruses. We have (1) identified a novel ebolavirus in bats in Sierra Leone that has the potential to infect humans, (2) expanded the known geographic range of Marburg virus to West Africa, and (3) found evidence of EBOV in a cave-dwelling bat in Liberia.

PREDICT detected 1 novel filovirus
and 2 known filoviruses.

Paramyxoviruses include well-known human viruses like measles and mumps. This group also includes zoonotic viruses like Nipah virus, which comes from fruit bats and causes outbreaks of fatal encephalitis in humans in places like Bangladesh and India. The PREDICT project has made many important contributions to our understanding of paramyxoviruses, including: (1) greatly expanding the known diversity of paramyxoviruses in bats and rodents, (2) supporting long-term surveillance for Nipah virus to understand the natural transmission dynamics of this virus in bats and the ecological drivers of spillover into humans, (3) providing virus genomes to the research community to support foundational work on virology and pathogenesis, and (4) the detection of OIE (World Organisation for Animal Health) notifiable livestock pathogens using our broad-spectrum cPCR detection approach.

PREDICT detected 192 novel paramyxoviruses
and 27 known paramyxoviruses.

Influenza viruses are well-known pathogens of both humans and animals. Both Influenza A and Influenza B were detected using PREDICT project protocols. Through our surveillance: (1) Influenza B was found in healthy people in communities and in people with respiratory illness seeking care at hospitals and clinics in 12 countries across Africa and Asia, (2) Influenza A subtypes H1N1 and H3N2 were detected in people, (3) surveillance in domestic livestock included detection of the highly pathogenic influenza viruses H5N1 and H9N2 in poultry, and (4) five subtypes of low pathogenic avian influenza viruses were found in wild birds along the flyway in Mongolia.

STRENGTHENING GLOBAL LABORATORY CAPACITY FOR VIRUS DISCOVERY & CHARACTERIZATION

NETWORK OF PARTNERSHIPS

One of the PREDICT project's major legacies and successes is the bolstering of laboratory capacity over the past ten years. Through coordination with 96 ministries in 36 countries, we improved testing capacity in 67 labs. One of our goals was to strengthen virus detection capabilities, in particular for the priority virus groups. In 2009, when we first began working with laboratory partners, we identified infrastructure improvements and safety procedure needs. Over time, laboratory protocols and control materials were provided along with training and guidance to implement virus detection protocols. Beyond training for sample testing, result tracking, and data management, we also focused on result reporting and data sharing across government sectors, international partners, and other stakeholders. Over the lifespan of the project, we forged an international network between laboratories and government ministries and our teams became a trusted resource and "go to" partner for questions and assistance for testing needs along with outbreak support.

ONE APPROACH ACROSS SPECIES

Laboratory tests aid in the detection of a virus and diagnosis of disease. Testing, when targeting relevant samples based on the type of contact between the human and animal groups, can provide information on the potential route(s) of exposure to the virus and the environmental circumstances that may promote disease. While many projects use traditional testing, which allows for detection of one or more specific viruses in a certain host, this approach makes it difficult to compare results across host species and different testing methods. To detect known, unknown, and emerging viruses in varied species, a shift in strategy was needed to be widely applicable. Using cPCR targeting specific virus groups allowed for detection of known and unknown viruses in all host species and sample types. Employing these methods in human and animal health laboratories is a more sustainable approach to discovery of novel viruses, and these methods are helpful tools for the detection of mystery illnesses affecting multiple hosts.

Using our strategy, from 2014-2019 our teams tested 208,016 samples from 109,154 people, domestic animals, and wild animals. As a testament to major capacity gains and global health security, 86% of the testing was performed by our collaborating labs in partner countries, and these local labs detected 74% of all viruses identified in this phase of the project.

THE RIGHT TOOL FOR THE RIGHT JOB

SPECIFIC PCR	**DETECTS**: Specific virus **USES**: Specific surveillance, rule in/out outbreak cases
CONSENSUS PCR	**DETECTS**: Known and previously unknown viruses **USES**: Virus discovery, surveillance, new outbreak identification

HOW CAN VIRUS DISCOVERY HELP US UNDERSTAND ZOONOTIC RISK?

Finding new viruses in wildlife will not stop spillover, at least not yet and not directly. However, it *can* help us evaluate the zoonotic potential of specific viruses circulating in proximity to humans *and* help us understand the mechanisms by which wildlife-origin viruses evolve and acquire the ability to infect people in the first place. Here, we show how virus discovery can be used to better understand zoonotic risk at different scales – from the risk posed by specific viruses in specific regions, to the ecological and evolutionary mechanisms shaping zoonotic risk for many viruses across geographic scales. The virus discoveries made possible through cPCR were used to support epidemiological and ecological studies that identified risk factors important for spillover, while HTS data supported experimental in vitro studies and evolutionary studies aimed at assessing zoonotic threat and zoonotic potential.

EVALUATING ZOONOTIC RISK: EPIDEMIOLOGICAL & ECOLOGICAL STUDIES

NEWFOUND AFFINITY: CORONAVIRUSES & BATS

Consensus PCR generates population-level data on virus diversity, host and geographic range, and shedding patterns that can be used to understand ecological and epidemiological aspects of zoonotic risk. In a survey of 88,233 animals and humans, we identified 177 unique coronaviruses (Table 2) and used these data to show (1) that bats are significantly more likely to carry coronaviruses than other species, (2) that there are likely to be over 3,700 different types of coronaviruses yet to be discovered in bats, (3) that coronaviruses are primarily found in the gastrointestinal tract of bats, and (4) that different types of coronaviruses have strong geographic and host associations (Anthony et al. 2017a). These findings inform on zoonotic risk and can be used to guide decision-making and future surveillance. For instance, we used these findings to predict the geographic range of bat SARS-related viruses (Figure 2) and to target community-based interventions towards people with high-risk exposure to bat feces (Figure 3).

TABLE 2. ***Coronavirus surveillance effort throughout the PREDICT project (2009-2020)***

HOST TAXA TESTED	# INDIVIDUALS TESTED	# INDIVIDUALS POSITIVE	# DISTINCT VIRUSES DETECTED
BATS	38,240	2,984	148
NON-HUMAN PRIMATES	9,908	4	2
RODENTS/ SHREWS	18,090	817	18
OTHER WILDLIFE	1,963	25	4
HUMANS	16,101	73	4
DOMESTIC SPECIES	3,931	321	9
TOTAL	**88,233**	**4,224**	**177***

*Note: Numbers do not total as some viruses were detected in more than one taxa

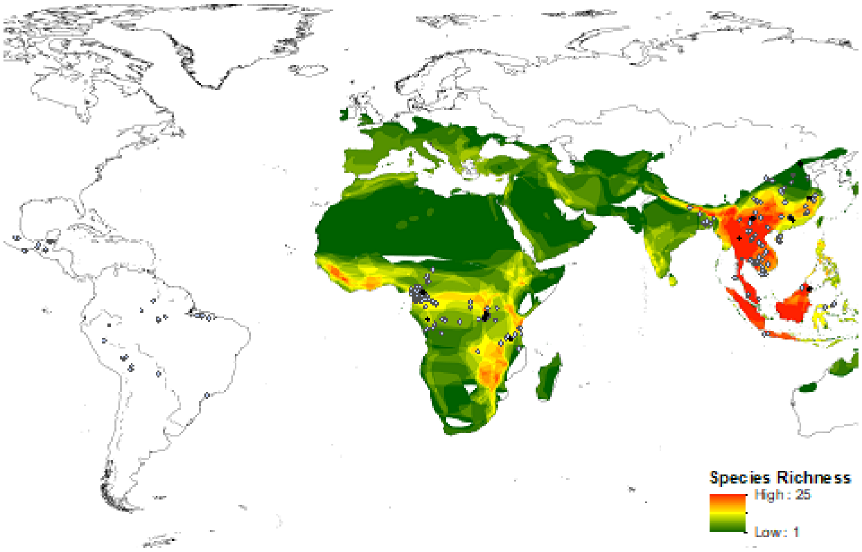


FIGURE 2. ***Map showing the predicted hotspots for coronaviruses within the Sarbecovirus subgenus. This group includes viruses like SARS-CoV-1 and SARS-CoV-2. Dots indicate places where PREDICT found coronaviruses (2009-2014). Black dots indicate sarbecoviruses specifically.***

FIGURE 3. ***Community engagement in Cambodia where our team worked with communities identified as high-risk for coronavirus infections from bats.***

EXPOSING SPILLOVER: EBOLAVIRUSES

When new viruses are detected or previously known viruses emerge in new places and cause illness, evaluating risk in terms of ecological, behavioral and epidemiologic data is often needed. Previous exposure to a virus can be assessed in order to determine how far back exposure may have occurred, understand how widespread exposure may be, and to investigate risk factors and human behaviors that may increase the risk of exposure to the disease. After the second largest Ebola virus disease (EVD) outbreak began in the Democratic Republic of the Congo (DRC) in July 2018 in North Kivu Province, we developed a new test to measure antibodies to ebolaviruses. Survey data from febrile patients seeking care in North Kivu Province prior to the EVD outbreak were evaluated to understand factors linked to ebolavirus exposure (Figure 4). In a survey of 272 people seeking care, we identified antibodies to ebolaviruses in 30 people. Through these data we (1) provide the first documented evidence of exposure to Ebola virus in people in Eastern DRC prior to the declaration of the 2018-2020 outbreak, (2) report the first known detection of antibodies to Bombali virus in a child, a new filovirus previously detected in bats in West and East Africa (see ***Special Feature: Ebola Host Project*** for more), and (3) link increased risk for exposure in women and children (Goldstein et al., *In Press*). Our data show that spillover from wild animals into humans is more frequent and geographically widespread than previously thought and support growing evidence that a range of severity of ebolavirus illness occurs in people.

> *Spillover from wild animals into humans is more frequent and geographically widespread than previously thought*

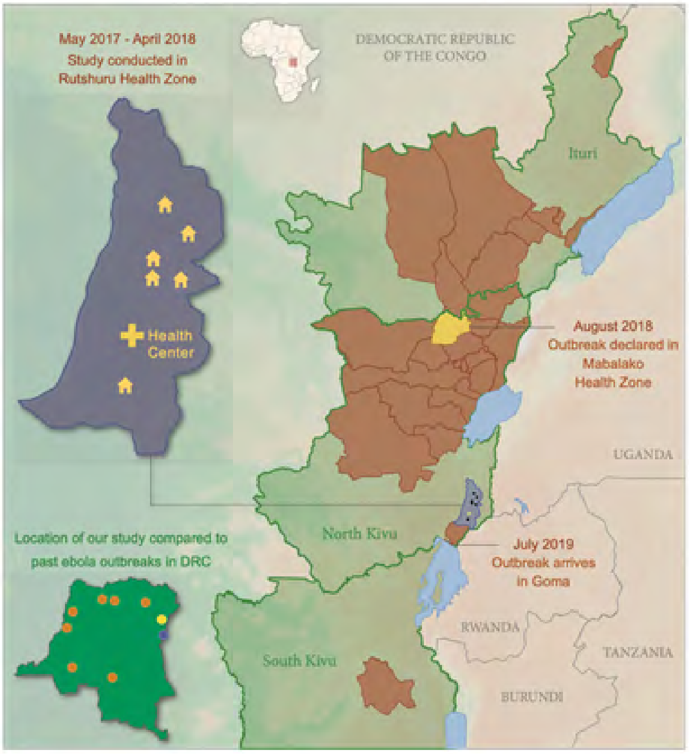


FIGURE 4. ***Villages and the Rubare Health Center in North Kivu Province where febrile patients traveled and were treated prior to the Ebola Virus Disease outbreak in Eastern DRC that began in 2018. The outbreak was first observed in the Mabalako Health Zone (yellow) in North Kivu before spreading to other areas (brown). Inset map of the DRC: Location of the 2018-2020 outbreak (yellow), study location (purple) and other EBOV outbreaks in the DRC to date (orange).***

EVALUATING ZOONOTIC RISK: EXPERIMENTAL IN VITRO STUDIES

It is extremely difficult to evaluate whether a novel virus discovered in wildlife poses a threat to humans. Infection success and any associated disease outcomes depend on hundreds of interactions between the virus and the host cell, and this complexity can be extremely difficult to recapitulate and predict in the lab. Nonetheless, there are a series of key barriers that a virus must be able to overcome in order to pose a zoonotic threat. First, it must be able to enter human cells by interacting with cell-surface receptor proteins. Then, it must be able to interact with human cellular machinery and be able to replicate efficiently once inside. Finally, it must be able to block the human immune response (e.g. interferon synthesis and release) to avoid being destroyed before it can replicate and transmit to a new host. To better understand the zoonotic potential of wildlife viruses, we used different experimental approaches to evaluate the ability of certain viruses to (1) enter human cells, (2) replicate efficiently, and (3) block the human interferon response.

THE DEVIL IS IN THE DETAILS: CORONAVIRUS SPIKE GENE

We identified a novel coronavirus in bats from Uganda that is closely related to human Middle East respiratory syndrome coronavirus, MERS-CoV. The virus was named PDF-2180, and genetic analysis showed that it was closely related to MERS-CoV across much of its genome – except for one important region, the spike gene (Figure 5). The spike gene contains the data necessary to synthesize the spike protein, which mediates virus entry into cells; therefore, changes in the spike gene sequence could alter which cells and host species a virus can infect. If PDF-2180 differs from MERS-CoV in the spike gene, it raises important questions about whether PDF-2180 can infect humans. Indeed, using recombinant virus approaches, we showed that PDF-2180 is not likely able to mediate infection of human cells using the same entry receptor as MERS-CoV. Thus, despite being closely related to MERS-CoV, the PDF-2180 bat virus does not appear to pose a threat to humans at this time. This work is an important reminder that even though two viruses might be closely related, the zoonotic risk they each pose could be very different (see *Recombination and Zoonotic Potential* for more on this point).

> *Even though two viruses might be closely related, their zoonotic risks could be very different*

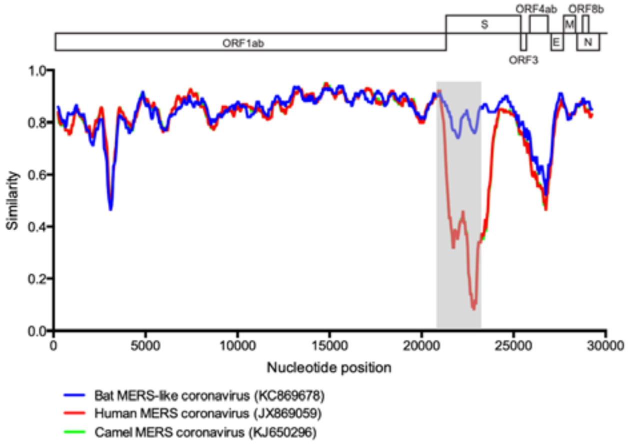


FIGURE 5. ***Simplot showing genetic similarity of PDF-2180 to a bat MERS-like CoV, and to MERS-CoV isolates from humans and camels (Anthony et al. 2017b). The PDF-2180 virus is the reference. Sequence similarity is plotted on the Y axis; a similarity of 1 indicates identical sequences. All three viruses show high similarity to PDF-2180 in ORF1a/b gene. In the spike gene (S), similarity between human/camel MERS-CoV and PDF-2180 drops significantly, while similarity to the bat MERS-like virus remains high. This pattern suggests a recombination event may have occurred.***

EFFICIENCY IS KEY: BOMBALI VIRUS

We identified a novel species within the *Ebolavirus* genus, Bombali virus (species: *Bombali ebolavirus*; BOMV), in two species of insectivorous bats (free-tailed bats, family Molossidae) in Sierra Leone (see *Special Feature: Ebola Host Project* for more). The bats were collected inside people's houses, which is concerning because ebolaviruses can be highly pathogenic and because the proximity of this new virus to humans suggests there is opportunity for spillover to occur. Ebolaviruses enter host cells by attaching to a host receptor protein called NPC1, so we evaluated the ability of BOMV to attach to human NPC1 to determine whether this virus poses any risk to humans. We showed that BOMV can indeed bind to human NPC1 and enter human cells, but also found that BOMV is less efficient at doing this compared to Ebola virus (species: *Zaire ebolavirus*; EBOV; Figure 6). Because BOMV has the potential to infect human cells, albeit less efficiently than EBOV, we moved to evaluate other key traits, such as the ability of BOMV to block the interferon response once inside the cell. We showed that BOMV was able block some parts of the human interferon response, but again found that it was not as efficient at doing so, compared to EBOV. Overall, this work suggests that BOMV might have zoonotic potential; however, lower efficiency in entry and interferon antagonism suggest that it may not pose a serious disease threat.

PHOTO: JABER BELKHIRIA

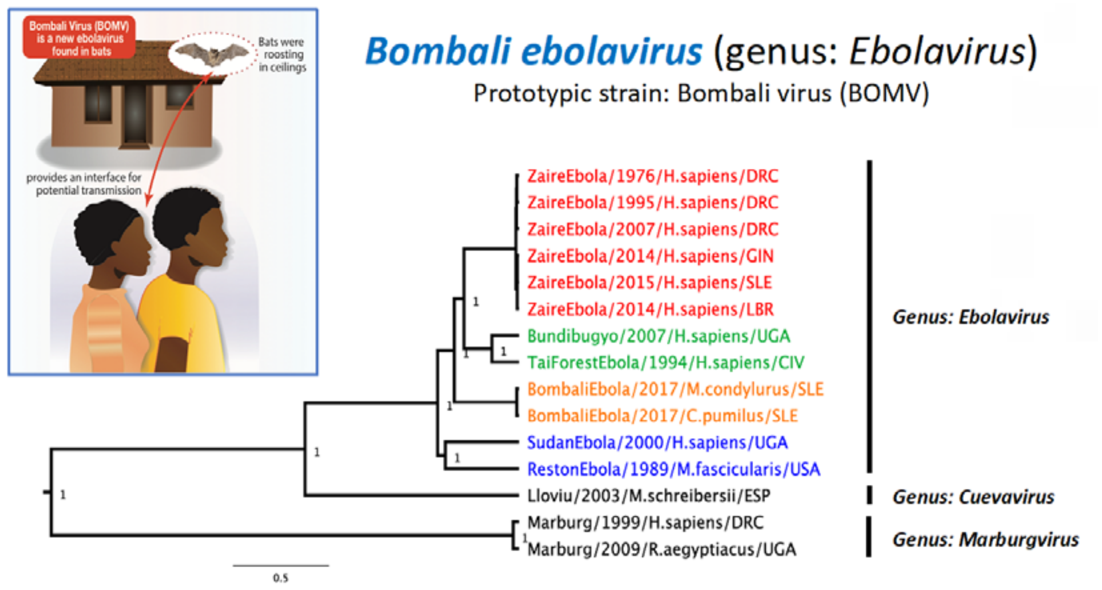


FIGURE 6. *A novel ebolavirus, Bombali virus (BOMV; orange), was discovered in bats in Sierra Leone (Goldstein et al. 2018). This virus has been assigned as the type strain of a new species (***Bombali ebolavirus***) within the genus Ebolavirus. BOMV was first discovered in insectivorous bats belonging to the species* **Mops condylurus** *and* **Chaerephon pumilus** *(family: Molossidae). It has since also been detected in* **M. condylurus** *bats in Guinea and Kenya. Experimental evidence confirms that the BOMV glycoprotein can mediate entry into human cells, and there is serologic evidence of human exposure in the Democratic Republic of the Congo.*

UNDERSTANDING EMERGENCE: EVOLUTIONARY STUDIES

Evolutionary studies can give us insights into the natural history of wildlife viruses and nicely complement experimental studies focused on evaluating the zoonotic potential of specific viruses. They can help identify evolutionary events that explain how viruses come to be what they are today and why some have the potential to infect humans while others do not. They provide context for the origins of zoonotic viruses (e.g. host or geographic origins) and provide insights into the mechanisms and pathway for emergence in humans.

RECOMBINATION & ZOONOTIC POTENTIAL: CORONAVIRUSES

Recombination is an evolutionary mechanism that allows coronaviruses to swap out genes or gene fragments when a second virus is present in the same cell at the same time. If a coronavirus acquires a new version of the gene that helps it enter host cells, it can dramatically alter the types of cells, and thus species, that it can infect. Using a combination of comparative genomics and laboratory studies, we showed that recombination has had a significant impact on the emergence of coronaviruses in humans (Anthony et al., 2017; Wells et al., *submitted*). We showed that both MERS-CoV and SARS-CoV-1 are recombinants and did not originally have the ability to infect humans. Indeed, both MERS-CoV and SARS-CoV-1 likely acquired new spike genes by recombining with related bat viruses that already had the genetic ability to enter human cells. In the case of SARS-CoV-1, we showed that it actually acquired a human-compatible spike gene from a virus within the same evolutionary group that includes SARS-CoV-2, the virus that causes COVID-19. We showed that while SARS-CoV-2 has long had the ability to infect humans, SARS-CoV-1 acquired this trait more recently by recombining with a virus closely related to SARS-CoV-2. This work is another reminder that closely related viruses can be very different in terms of the risk they pose to humans (because of recombination) and underscores the role of evolutionary mechanisms in shaping the zoonotic potential of coronaviruses.

> *Recombination has had a significant impact on the emergence of coronaviruses in humans*

TARGETED SURVEILLANCE FOR SPECIFIC, KEY PATHOGENS

This is the first time EBOV RNA has been found in a bat in West Africa

Targeted virus surveillance, combining specific virus testing and broad virus group testing, can be used to (1) identify animal hosts and reservoirs for known viruses, (2) better understand the geographic distribution of viruses and timing and seasonality of viral shedding, and (3) determine the potential route(s) of transmission of viruses that can cause epidemic diseases. Over the course of the PREDICT project, we conducted multiple studies focused on the surveillance of specific pathogens to try to understand more about the epidemiologic risk factors and ecological drivers of spillover and emergence in humans. Here, we share three deep dives into our work characterizing filovirus host and geographic range (see *Special Feature: Ebola Host Project* for more).

Public health authorities now recognize the need to include MARV as part of natural surveillance plans for filoviruses

LESSONS LEARNED FROM FILOVIRUS SURVEILLANCE

FINDING A NEEDLE IN A HAYSTACK: EBOLA VIRUS IN WEST AFRICA

The 2013-2016 West Africa Ebola epidemic was an important reminder of the significant public health and economic impact of Ebola virus (EBOV; species, *Zaire ebolavirus*). Yet despite more than four decades of human outbreaks and research, the natural reservoir(s) of Ebola virus remain unknown. As a result, we know almost nothing about the transmission dynamics of this virus in its natural host(s) and remain unprepared to mitigate the risk of future outbreaks. To further our understanding of EBOV host range, we conducted expansive surveillance of bats and other wildlife in Libera, Guinea, and Sierra Leone.

Between September 2016 and August 2018, 11,742 samples from 5,327 animals (majority were bats) were collected from 26 sites across Liberia including Nimba, Lofa, Montserrado, Bong, and Grand Cape Mount counties. Samples were tested using four assays targeting filoviruses, including a filovirus group assay, a general ebolavirus assay, and two tests specific for the strain of EBOV that caused the outbreak in West Africa. A single insectivorous bat, a Nimba long-fingered bat (*Miniopterus nimbae*) from Liberia's northeastern Nimba District (Figure 7), was positive for EBOV. Genetic sequence of the EBOV genome was recovered from the saliva samples (20% of the genome) and showed >99% sequence identity to the West African strain that emerged in humans. Neutralizing antibodies were also detected in the serum of the EBOV positive bat.

This detection represents the first time EBOV RNA has been found in a bat in West Africa. Further surveillance for EBOV in this species is now needed to investigate whether this result was an anomalous finding (e.g. spillover from another bat species cohabiting in the same cave) or if *M. nimbae* bats contribute to the natural ecology of EBOV.

Distribution of filoviruses in nature is more widespread than previously recognized

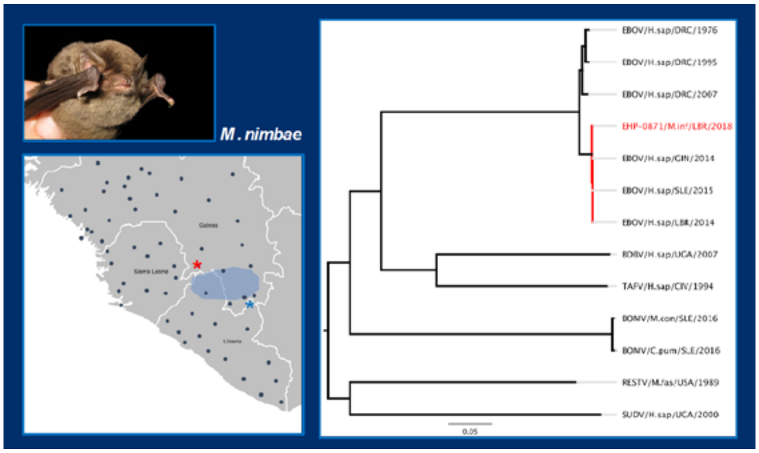


FIGURE 7. ***(Left) Location of the Ebola virus positive Nimba long fingered bat (*) and the location of the origin (*) of the 2013-2016 outbreak. (Right) The Ebola virus sequence from the bat (red tree branch) was most similar to the strains found in people that died during the outbreak in Liberia, Guinea, and Sierra Leone.***

MORE WIDESPREAD THAN PREVIOUSLY KNOWN: MARBURG VIRUS IN WEST AFRICA

Marburg virus (MARV) is a cousin to EBOV (see Figure 6) and causes similar disease in people. There have been 12 MARV outbreaks reported; however, to date there have been no reported cases anywhere in West Africa.

Between March 2016 to October 2019, we collected 20,502 samples from 7,774 animals (majority were bats) at 25 sites in Sierra Leone. Colleagues from the US Centers for Disease Control also collected samples from Egyptian fruit bats (*Rousettus aegyptiacus*) at two additional locations. Samples were tested using three assays targeting filoviruses including a filovirus group assay, a general ebolavirus assay, and a specific test for MARV – just like our approach described above for EBOV surveillance. Eleven Egyptian fruit bats from three caves were positive for MARV, which represents the first detection of this virus in West Africa. Sierra Leone is >2,400 km from the nearest known MARV-positive site in Africa (Figure 8), showing that MARV is far more widely distributed than previously recognized. Based on this finding, public health authorities now recognize the need to include MARV as part of natural surveillance plans for filoviruses.

WHERE YOU FIND THE BATS, YOU FIND THE VIRUS: BOMBALI VIRUS ACROSS AFRICA

After the discovery of BOMV in bats in Sierra Leone (see *Efficiency is Key: Bombali Virus* above), we developed a specific and more sensitive test for the new virus. BOMV was first identified in five insect eating bats in the Bombali district in May of 2016. We tested an additional 27,112 samples from 9,851 animals sampled from September 2016 to May 2019 in Guinea and Sierra Leone. Two Angolan free-tailed bats (*Mops condylurus*) were positive in Guinea in the districts of Guéckedou and Kissidougou in May and June of 2018 (Figure 9), and two additional bats were found to be positive in the Bombali district in Sierra Leone. Concurrently, BOMV was also detected in an Angolan free-tailed bat by another research group in Kenya in May 2018 (Forbes et al., 2019), highlighting how our discoveries and diagnostic assays are being used to support surveillance beyond the bounds of the PREDICT project. Evidence of BOMV in bats from Sierra Leone, Guinea, and Kenya shows that the virus has a very broad geographic distribution. Coupled with our MARV findings, it shows that the distribution of filoviruses in nature is more widespread than previously recognized.

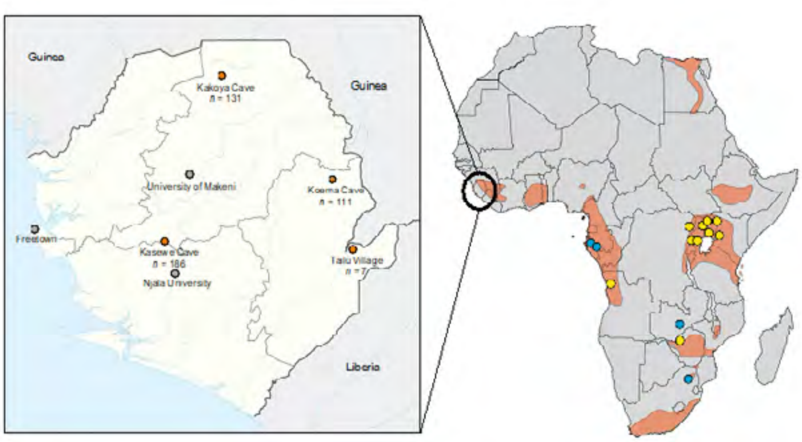


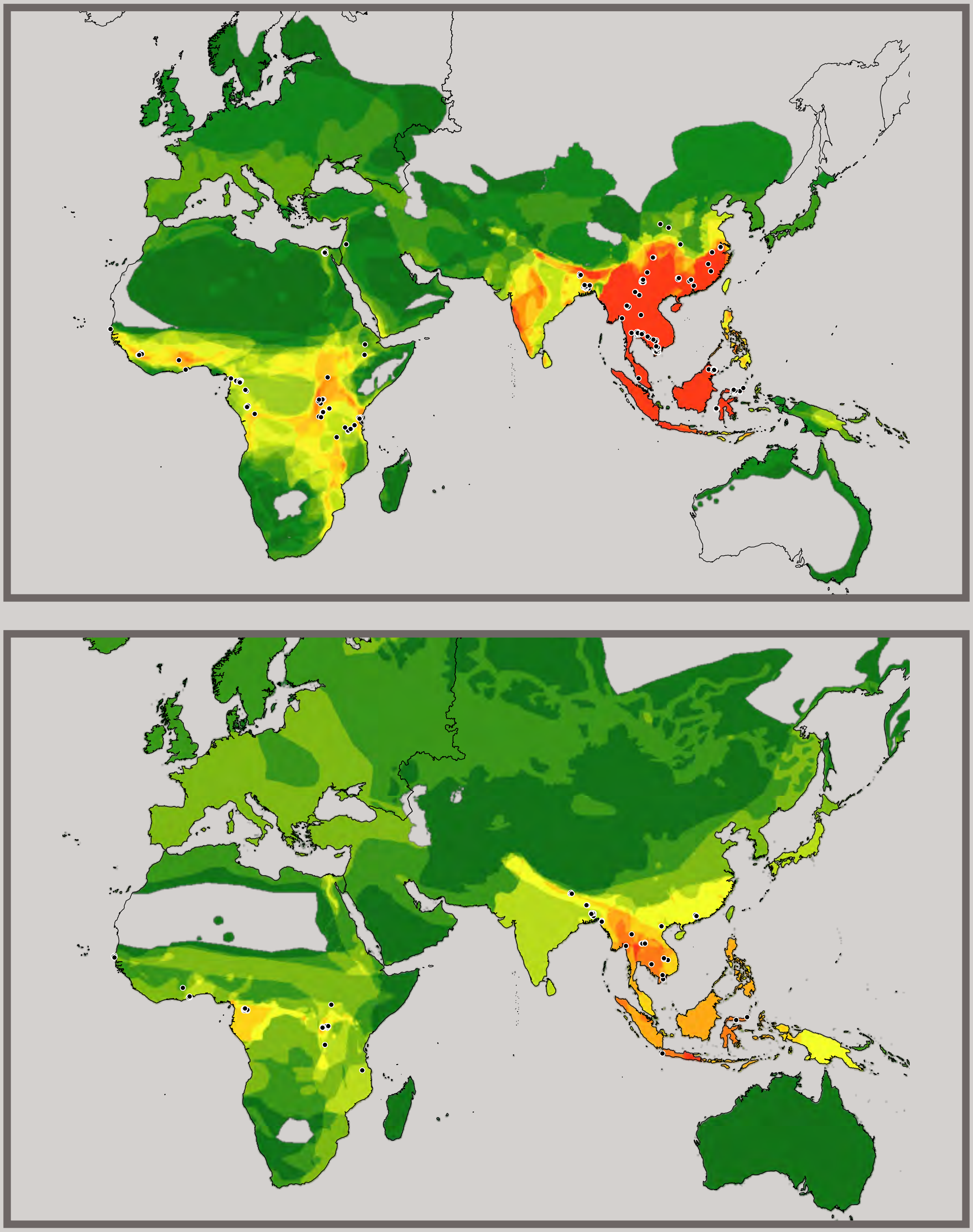
FIGURE 8. *Caves where Marburg virus (MARV) was detected in Egyptian fruit bats in Sierra Leone (orange circles). Locations in other parts of Africa where MARV has been detected in bats without outbreaks (blue circles), with known MARV outbreaks (yellow circles), and the geographic range of Egyptian fruit bats, the natural host reservoir of MARV (orange shaded) (Amman et al. 2019).*

FIGURE 9. *Bombali virus has been found in Little free-tailed bats* (**Chaerephon pumilus**) *and Angolan free-tailed bats* (**Mops condylurus**) *in Sierra Leone, Guinea, and Kenya. These widespread countries are within the geographic ranges for these host species.*

SPECIAL FEATURE

PARAMYXOVIRUS DISCOVERY

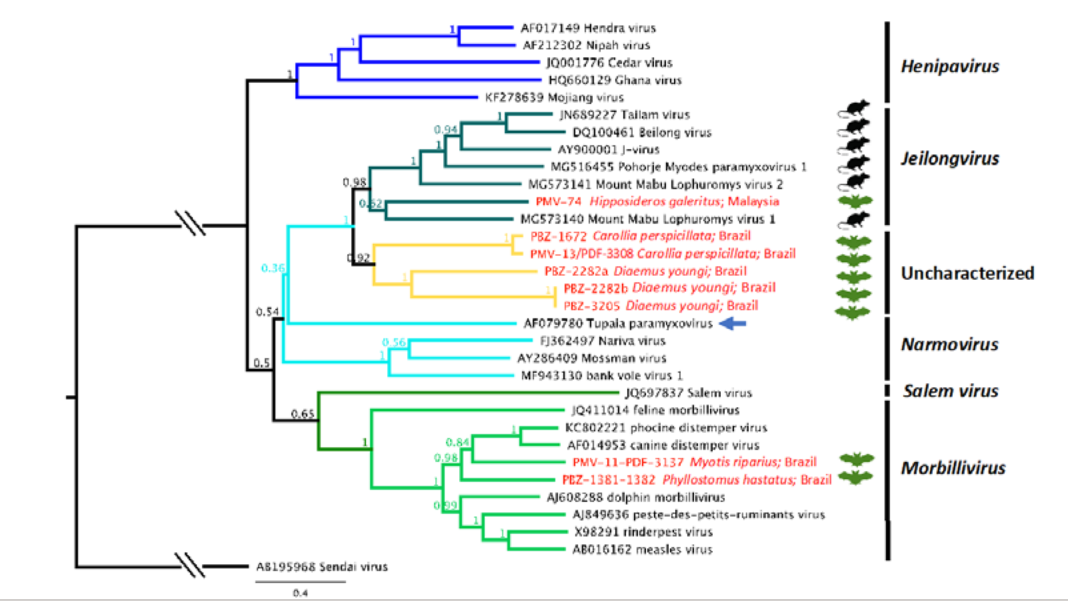
The PREDICT project has greatly expanded our knowledge of paramyxovirus (PMV) diversity with the discovery of 192 novel paramyxoviruses in a variety of hosts around the world. Knowledge of paramyxovirus biogeography has also been furthered, as indicated by the expansive range that these known and novel paramyxoviruses potentially occupy throughout Africa and Southeast Asia as a consequence of their host ranges (e.g. bats and rodents).



A heatmap of host species distributions (top – bats; bottom – rodents) that were positive for paramyxoviruses using the PREDICT consensus PCR approach (warmer colors indicate more hosts in that area). Dots represent paramyxovirus detections in these hosts.

Additionally, by analyzing the full genome sequences for eight of these paramyxoviruses, we have gained the following insights into paramyxovirus ecology, evolution, and taxonomy:

1. **Expansion of host range within a genus**: Until now, the virus genus *Jeilongvirus* only included paramyxoviruses found in rodents. We expanded the known host range and host diversity of viruses in this group with the discovery of PMV-74 in bats from Malaysia.
2. **Proposed new genus within the paramyxovirus group**: Several viruses discovered through our surveillance efforts appear to form a completely new genus of paramyxoviruses. This currently uncharacterized group is made up of bat viruses from Brazil, such as PMV-13.
3. **Reclassification of known viruses**: Tupaia paramyxovirus is a known paramyxovirus and was recently classified within the *Narmovirus* genus. The discoveries we made have revised our understanding of its phylogenetic placement in the paramyxovirus tree. The new phylogeny suggests it might be better classified as the prototypic virus of a separate (new) genus.
4. **Discovery of novel morbilliviruses**: Paramyxoviruses within the genus *Morbillivirus* tend to be highly pathogenic and are therefore of great significance to public and veterinary health. We discovered two novel morbilliviruses in bats from Brazil.



Phylogenetic tree of paramyxoviruses. Only a subset of paramyxovirus groups is shown. Full genome sequences of viruses discovered by the PREDICT project are shown in red, and host species and country of detection are indicated. Branches are colored according to current ICTV classification of genera.

PHOTO: TRACEY GOLDSTEIN

FUTURE DIRECTIONS

Although a relatively simple procedure, the use of cPCR for virus discovery and characterization was instrumental to the success of the PREDICT project. Already, these PREDICT-developed cPCR protocols have been adopted by independent research teams with positive results (see ***Where You Find the Bats, You Find the Virus: Bombali Virus Across Africa***). Moreover, our cPCR assays, targeting specific virus groups, enabled our partner labs in Cambodia, Thailand, and Nepal to detect SARS-CoV-2 in humans early on in the COVID-19 outbreak, *before* the complete virus genome was available and *before* the development of SARS-CoV-2 specific tests. The COVID-19 pandemic has turned a new page in human history – perhaps now, more than ever, the need for continuing virus discovery efforts is realized. We believe the PREDICT project has served as a proof-of-concept for virus discovery and characterization and has provided a solid platform for the continuation of these efforts.

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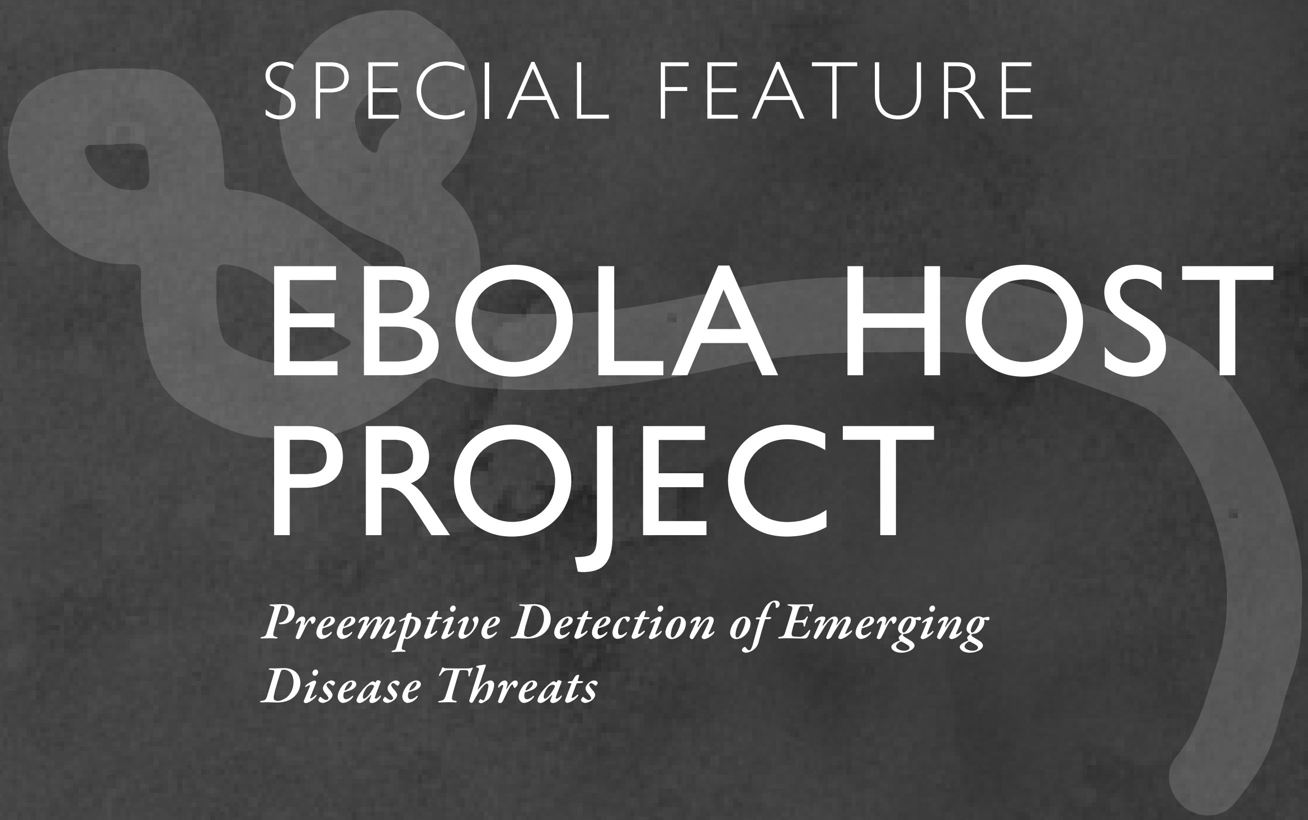


SPECIAL FEATURE

EBOLA HOST PROJECT

Preemptive Detection of Emerging Disease Threats

THE SEARCH BEGINS

In 2016, USAID and the PREDICT Consortium designed and implemented the Ebola Host Project (EHP) – the most comprehensive search for the animal origins of ebolaviruses and other closely related viruses since Ebola first emerged in Africa in 1976. Ambitious in both scope and scale, EHP succeeded in revolutionizing our understanding of these viruses and changed the map of their distribution across Africa. Our efforts led to the discovery of an entirely new species of ebolavirus (*Bombali ebolavirus*) in bats in Sierra Leone (Goldstein et al., 2018) and Guinea (Karan et al., 2019) and the detection for the first time in West Africa of the highly-lethal Marburg virus in bats in Sierra Leone (Amman et al., 2020). Both of these viruses were discovered and detected before an outbreak occurred in humans or animals, demonstrating that preemptive surveillance for viral threats can provide an early warning to help us prepare risk reduction and disease prevention strategies. In Liberia, the PREDICT team detected *Zaire ebolavirus* in a bat (Epstein et al., 2019), marking the first time that this virus was detected in a potential host species in West Africa. These discoveries serve as reminders that the cause of the 2014 West Africa Ebola epidemic and other potentially lethal filoviruses continue to lurk in the wild awaiting opportunities for reemergence and devastating outbreaks.

ALL PHOTOS IN THIS SPECIAL FEATURE: SIMON TOWNSLEY

EBOLAVIRUSES

ELUSIVE ADVERSARIES

Since Ebola virus first emerged in 1976, scientists have been on a quest to discover the animal source of the virus. By knowing how people come into contact with and get infected by this deadly virus (by interacting with the specific wild animal host(s)), we remove the element of surprise, allowing communities to develop informed strategies to reduce risk of spillover and prevent outbreaks. This search seems straightforward, but in more than 40-years of scientific study, dozens of Ebola outbreaks, the discovery of six different ebolavirus species and after thousands of lives lost, there is still no definitive and conclusive evidence that pinpoints a single animal species as Ebola's primary reservoir host. As Ebola continues to emerge in new and never before detected regions across the continent of Africa, it challenges our assumptions of where it comes from, how it moves around, and when it will next appear.

Research over the years has provided tantalizing clues, suggesting that bats and non-human primates are key players in the transmission of this zoonotic virus and are key links to human infections, but these clues came at too high a price. The five species of ebolavirus discovered between 1976 and 2007 were identified only *after* they spilled over into humans or animals, causing severe disease and death. However, as devastating as these outbreaks were, they largely occurred in relatively remote areas, either burning out naturally or being effectively contained through public health response efforts. Then just as mysteriously as it emerged, the ebolaviruses would disappear once again to be harbored by its mysterious hosts. This cycle would continue for 40 years until the magnitude of Ebola as a threat to global health security was laid bare in West Africa.

In December 2013, a two-year-old boy in southern Guinea, West Africa, died within four days of falling ill to an unknown illness. It would take another three months for health officials to identify the culprit – *Zaire ebolavirus*, the very same species of virus discovered in 1976, more than 5,000 km away in Central Africa. The death of this young boy would begin the largest Ebola virus outbreak in history, spreading across Guinea, Liberia, and Sierra Leone and infecting an estimated 28,646 – killing at least 11,323 people.

Beyond the direct local impacts, for the first time Ebola would threaten health security around the world, infecting individuals from seven additional countries including Nigeria, Spain, the United Kingdom, and the United States. Across the region and the world, emergency outbreak response efforts were stretched to the limits of capacity.

The World Health Organization declared the outbreak a Public Health Emergency of International Concern in mid-2014, and after two years of massive response efforts and the dedication of local and global health professionals and community activists, the epidemic was declared over in June 2016. However these efforts came at great cost, with the tragic deaths of 513 health care workers, the near collapse of local health services delivery and the regional economy, and $3.6 billion of direct foreign investment to fund the response efforts.

The epidemic raised important fundamental questions regarding this previously rare disease. How did *Zaire ebolavirus* travel from Central Africa to West Africa? Was it always there, hidden in an unknown host and spilling over for the first time in 2013? Or was 2013 simply the first spillover event that was finally recognized and reported? What made this outbreak so much bigger and deadlier than all the others? For perspective, the West Africa epidemic was 90 times larger than the first recognized Ebola outbreak in 1976 and had 10 times more confirmed cases than all previously known outbreaks combined. To understand this historical event and to prevent it from happening again, the global community launched several new initiatives to find *Zaire ebolavirus* in the wild and identify the wildlife hosts of this deadly pathogen; one of these initiatives was launched by PREDICT.

90 TIMES LARGER THAN THE FIRST EBOLA OUTBREAK IN 1976

> ***"The detection of Marburg virus in Sierra Leone increased our understanding of the distribution of this high-consequence virus, and by detecting it in the Egyptian fruit bat, we could be proactive – building coordinated strategies to inform the public of the health threat, show people how to live safely with bats, and significantly reduce the risk of an outbreak. This is exactly what PREDICT and the Ebola Host Project set out to do and is a major leap forward in our ability to prevent pandemic threats."***

Dr. Jonna Mazet,
Principal Investigator & Global Director (2009-2019), PREDICT, UC Davis

HOW RARE IS THE DETECTION OF EBOLA IN WILDLIFE?

During the first five years of PREDICT (2009-2014), our team tested over 5,000 bats (80+ suspected host species), 800 primates, and 600 rodents and shrews across seven countries in Africa, but we found no positive animal for *Zaire ebolavirus* or other closely related filoviruses, despite using the same virus detection platform that simultaneously detected so many other known and new viruses in these animals (see *Chapter 2*). Many of the animals we sampled and tested were believed to be possible filovirus hosts. Further, we targeted sample collection efforts in several known zoonotic virus hot spots and areas of previous Ebola outbreaks, such as Democratic Republic of the Congo and Uganda. However, we failed to detect Ebola or any other filovirus in our early efforts.

To proactively prevent spillover of *Zaire ebolavirus* and other related filoviruses, you first need to find their animal reservoir or sources; understand the animals' behavior, as well as that of people who interact with it; and then learn how, when, and where spillover risk is greatest. Equipped with this knowledge, you can then target interventions at exactly the right place and time. PREDICT's Ebola Host Project (EHP) developed a strategy aimed at preventing another Ebola outbreak. It seemed ambitious but straightforward: 1) find the animal host(s) of the virus, 2) understand the critical interface and exposure pathways, and 3) work closely with local communities to shift attitudes and behaviors to mitigate the risk of spillover and transmission. In reality, however, implementing this strategy was exceptionally challenging, as very little was known about the exact animal source of the virus, and local communities were still reeling from the effects of the epidemic.

LESS THAN 1% CHANCE

NEEDED TO SAMPLE HIGHER NUMBERS THAN EVER BEFORE

Informed by our experience over the first five-years, we estimated that there was less than 1% chance of detecting *Zaire ebolavirus* in healthy wildlife. Therefore, we designed EHP as a large-scale comprehensive, multi-year, multi-country investigation in Guinea, Liberia, and Sierra Leone. Our target was sampling up to 54,000 animals in just three years. Because previous efforts had been unsuccessful, we aimed to sample as wide a taxonomic range of potential wildlife hosts as possible, across diverse ecological niches, and in higher numbers than ever before.

SAFELY CAPTURE, SAMPLE, RELEASE

THE WORLD'S LARGEST QUEST FOR EBOLA'S ORIGIN IN THE WILD

For our EHP field teams over the next three years the mantra was – safely capture, sample, release; safely capture, sample, release; safely capture, sample, release – and they succeeded nearly 20,000 times, with over 113,000 individual specimens collected from animals at 69 different sites in the three countries. As they worked to strengthen disease detection capacity for filoviruses in labs across the region, the One Health Institute Laboratory at UC Davis and Columbia University Center for Infection and Immunity also screened 45,563 specimens for filoviruses to make the tight timeline. By the end of the project in 2020, the EHP was the largest and most comprehensive survey of animals seeking the reservoir of ebolaviruses to date in any region of Africa and resulted in three major discoveries that revolutionized the world's understanding of filoviruses and their distribution across the continent.

HIDDEN IN PLAIN SIGHT

Samples from bats collected across Guinea, Liberia, and Sierra Leone resulted in the detection of one new and two previously known filoviruses (Bombali virus, Ebola virus, and Marburg virus), illuminating that the threat from this family of viruses to human and animal health was more widespread and complex than previously thought. These findings were a major achievement and proof of concept of the PREDICT project's core goal to strengthen capacity in endemic areas for surveillance and detection of zoonotic health threats before an outbreak in people or animals occurs.

The PREDICT project's discovery of *Bombali ebolavirus* established the sixth known species of ebolavirus. Remarkably this was the first time that a new type of ebolavirus was discovered before causing disease in people or animals – and proves that proactive surveillance for these rare but potentially high-consequence pathogens can enhance our ability to prepare and to develop risk reduction and intervention strategies. Because Bombali virus was newly discovered, the PREDICT team immediately launched efforts, which as of this publication remain ongoing, to characterize the threat it poses to people and other animals. Bombali virus was detected in Angolan free-tailed (*Mops condylurus*) and Little free-tailed (*Chaerephon pumilus*) bats that were sampled in and around homes in four villages across the Bombali District of Sierra Leone (Goldstein et al., 2018). Since this initial discovery, the virus has also been detected in Guinea by the PREDICT team, as well as by an independent team of researchers (Karan et al., 2019), and as far away as Kenya by another team using the PREDICT project's testing protocols (Forbes et al., 2019).

In just a few years, our knowledge and understanding of this group of viruses has expanded greatly, and we have redrawn the map of ebolaviruses on the African continent. Knowledge of the exact host species (Angolan and little free-tailed bats), their geographical distribution and ecological niche (a preference for roosting in trees and near or in people's homes), allows us to predict and assess the exposure risk that this virus poses from the regional to the household level. Equipped with this data, we can prepare strategies that prevent spillover of Bombali virus wherever these bats are found.

Another striking discovery of a known and deadly virus emerged from work conducted near four caves in northern, central, and eastern Sierra Leone. For the first time ever in West Africa, the well-known and deadly pathogen Marburg virus was detected in samples collected from the known bat reservoir, the Egyptian fruit bat (*Rousettus aegyptiacus*). Detected around the same time by both PREDICT's EHP team and scientists with the U.S. Centers for Disease Control and Prevention (CDC), the discoveries were the first time Marburg virus had been found outside of Central or Eastern Africa and vastly

> ***“The working conditions were very hard in the field. For bat sampling it was essential to work nights, very late, crossing swamps and climbing mountains just to find caves or holes in trees that bats inhabited!”***
>
> **Dr. Douokoro Kalivogui “Kalis”**
> **EHP Guineé, Field Coordinator**

expanded the range of this high-consequence pathogen to include all of equatorial Africa (Amman et al., 2020). With assistance from USAID and other agencies, this finding promoted the establishment of diagnostic capacity for this virus across the EHP country network, and clinicians became empowered with the knowledge that it is a potential differential diagnosis for cases of hemorrhagic disease.

Not long after these discoveries, PREDICT’s EHP team in Liberia detected and reported the first evidence of Ebola virus (*Zaire ebolavirus*) in West Africa in an insect-eating bat, the Nimba long-fingered bat (*Miniopterus nimbae*) (Epstein et al., 2019), marking the first time that evidence of the Ebola virus had been found in any bat in West Africa. It also represents the first detection of *Zaire ebolavirus* in any animal in the region since the end of the West African Ebola epidemic, an unsettling reminder that the virus is likely circulating among wildlife in the region and that human interaction with wildlife continues to pose a threat to human and animal health. As seen previously in Sierra Leone, the preemptive detection of this virus before the recognition of another human outbreak enabled PREDICT to work with the Government of Liberia to craft proactive risk reduction strategies, conduct robust community engagement and education campaigns, and further strengthen Liberia’s Ebola surveillance and detection capacity.

SIERRA LEONE (2018)

PREDICT reports discovery of an entirely new species of ebolavirus (*Bombali ebolavirus*) in insect-eating bats

PREDICT and US CDC report the detection of Marburg virus in fruit-eating bats in Sierra Leone, the first time in West Africa

LIBERIA (2019)

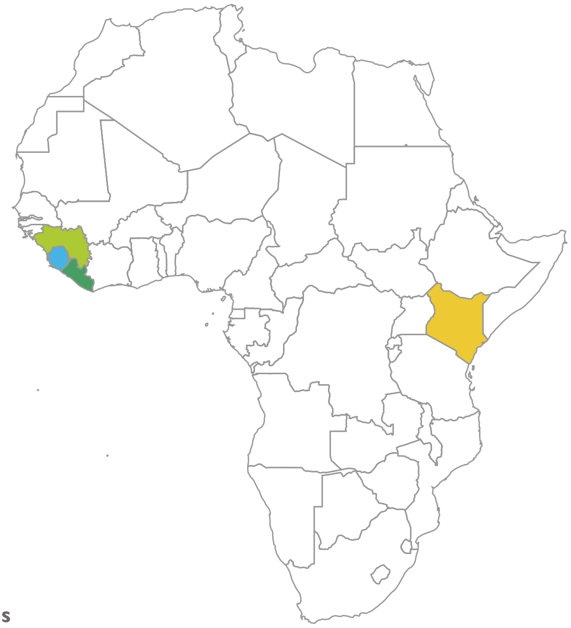
PREDICT reports that *Zaire ebolavirus*, the same ebolavirus species that caused the 2014 West Africa Ebola epidemic, has been detected in an insect-eating bat

GUINEA (2019)

***Bombali ebolavirus* discovered by an independent team, as well as by PREDICT in the same species of insect-eating bats as in Sierra Leone**

KENYA (2019)

An independent team using PREDICT virus detection protocols confirms *Bombali ebolavirus* in the same insect-eating bat species as first detected in Sierra Leone over 5,400 km away in East Africa – firmly establishing the apparent widespread distribution of multiple ebolaviruses in bats



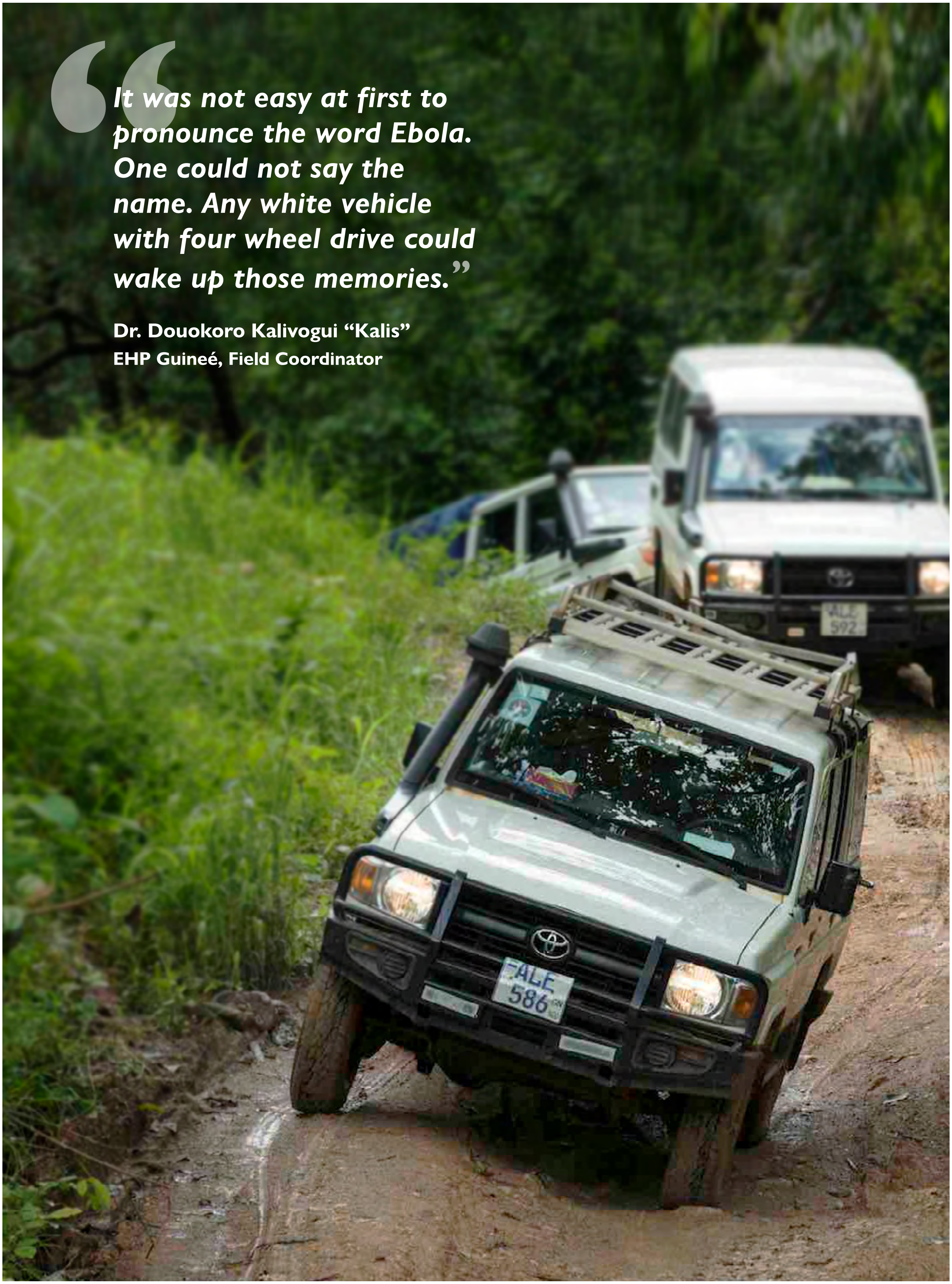
EVIDENCE + TRUST = EFFECTIVE RISK COMMUNICATION

What do you do after identifying dangerous viruses? The Ebola Host Project's discoveries had profound implications for national and global health security and required a sensitive and coordinated approach to health communications. Sensitivity was especially important due to the devastating effects of the recent Ebola epidemic on people's health and livelihoods in the region.

As with all PREDICT teams around the world, well before launching sampling efforts, our teams in Guinea, Liberia, and Sierra Leone conducted outreach and worked hand-in-hand to build partnerships with over 65 local communities, hundreds of traditional leaders, and multiple government partners from the national to district and local levels across the human, animal, and environmental sectors.

After establishing a basic level of trust, our PREDICT teams strived to continue dialogue to strengthen our engagement and relationships with all stakeholders. This strategy was essential to the project's success and allowed our teams to more safely and effectively carry out difficult wildlife sampling work. These partnerships would also prove critical following our significant virus discoveries, as the relationships and trust enabled us to launch successful health alert and risk-reduction communication campaigns. Following all of our discoveries, the PREDICT team, USAID, CDC, and Governments of Guinea, Liberia, and Sierra Leone coordinated massive public health outreach campaigns from the national level to the locally-affected communities. These campaigns were designed to raise awareness of the potential health risks posed by Bombali virus, Marburg virus, and Ebola virus along with other newly emerging potential pathogens in the region.

In each country, our teams worked with local government officials, health professionals, and community members to communicate clearly, accurately, and in a culturally appropriate manner the essential information for each discovery along with strategies to reduce the risk of transmission. Spurred initially by the discovery of Bombali virus, we created the *Living Safely with Bats* risk reduction and behavior change communication resource, which was first tested with trusted community leaders in Sierra Leone and then subsequently adapted into a more broad-based educational tool, which has been translated into 12 languages for use around the world (see ***Special Feature: Living Safely with Bats***).



> *It was not easy at first to pronounce the word Ebola. One could not say the name. Any white vehicle with four wheel drive could wake up those memories.*

Dr. Douokoro Kalivogui "Kalis"
EHP Guineé, Field Coordinator

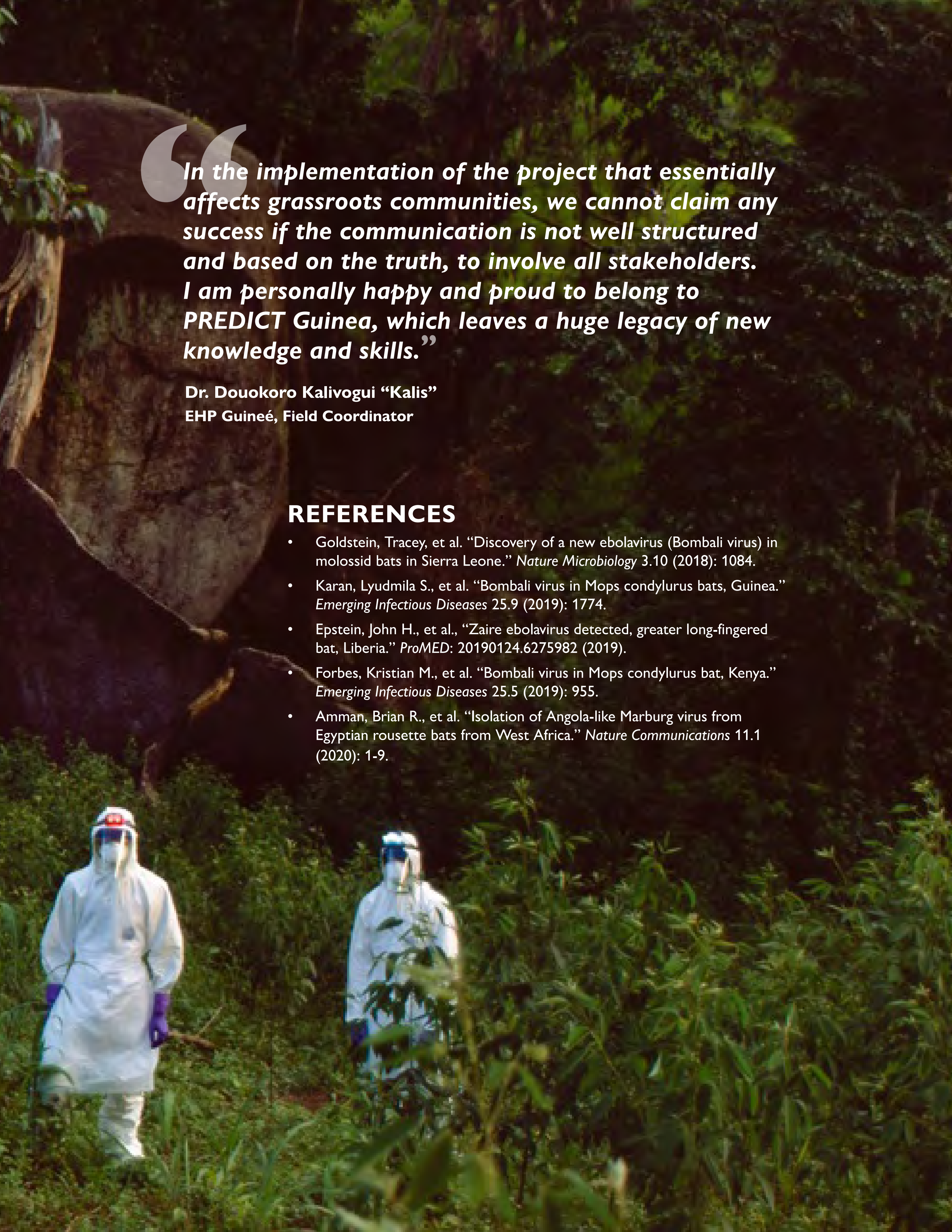
FROM EBOLA TO COVID-19

SCIENCE IS KEY TO ACHIEVING HEALTH SECURITY

PREDICT's Ebola Host Project is proof of concept that detecting viral threats before the first known spillover and launching proactive evidence-based interventions and risk reduction strategies can strengthen health security and prevent outbreaks. In the wake of COVID-19, EHP serves as a powerful model of the benefits of the One Health approach to disease prevention, detection, and response, especially at the interface of wildlife, people, and pathogens.

Capacity gains supported by the PREDICT EHP project in Guinea, Liberia, and Sierra Leone also helped prepare these countries to respond to other emerging health threats. Despite limited resources, as the COVID-19 pandemic raced around the globe, all three countries activated response strategies, some partly relying on former EHP staff and personnel to minimize the dangerous virus' impact (see Chapter 6). Through projects such as PREDICT and EHP, we have learned critical lessons on how best to search, detect, and identify looming pandemic threats, while building local capacities at all levels to be better prepared to prevent the next Disease X.

> ***"We hold PREDICT close to our heart. Scientists are few in this part of the world. Therefore, science should be key, as the more you know the better. This project captures the need for real science in secondary and university schools."***
>
> **Bai Shebora Kasanga II**
> **Paramount Chief of Bombali**



> *"In the implementation of the project that essentially affects grassroots communities, we cannot claim any success if the communication is not well structured and based on the truth, to involve all stakeholders. I am personally happy and proud to belong to PREDICT Guinea, which leaves a huge legacy of new knowledge and skills."*
>
> **Dr. Douokoro Kalivogui "Kalis"**
> **EHP Guineé, Field Coordinator**

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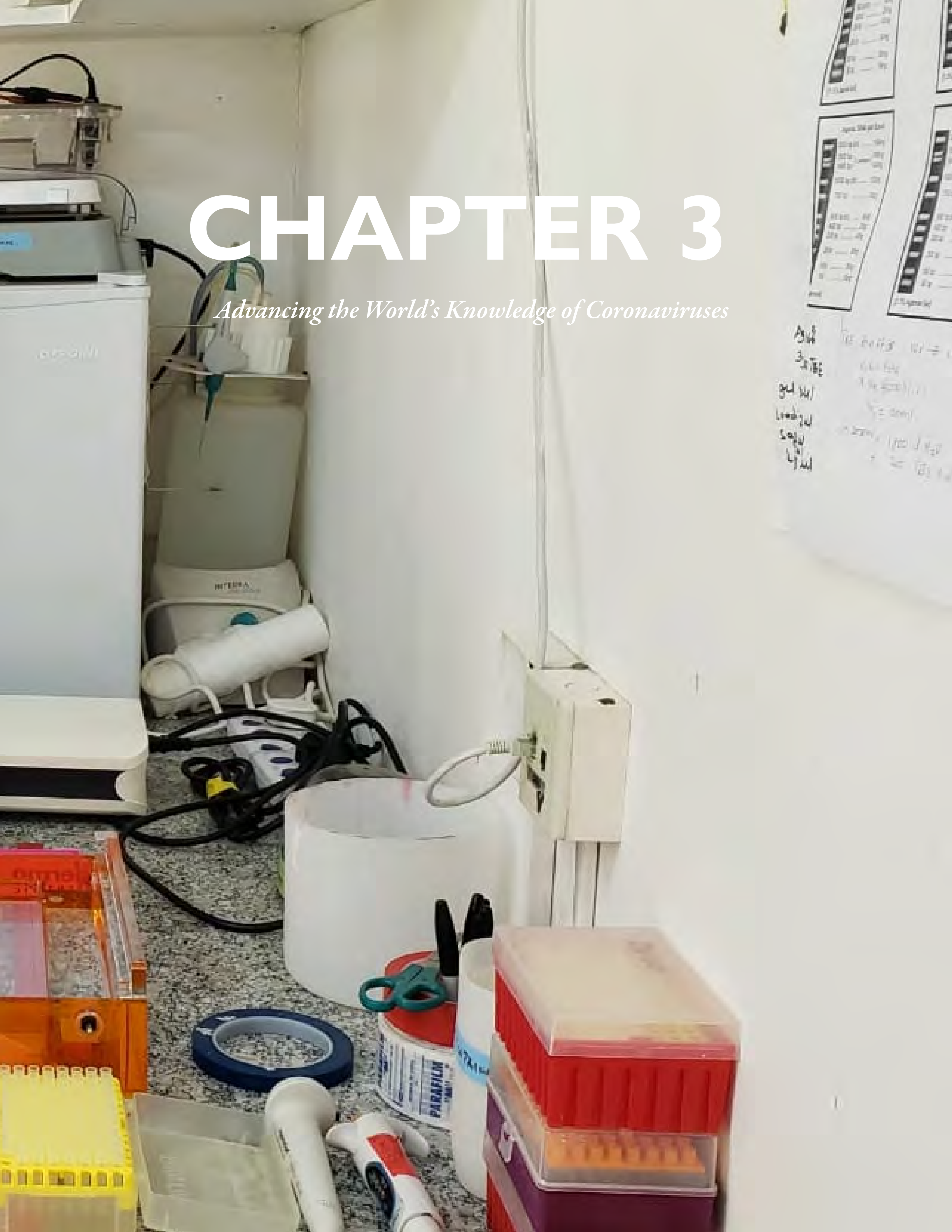
PHOTO: PREDICT TANZANIA

CHAPTER 3

Advancing the World's Knowledge of Coronaviruses



CORONAVIRUSES

A BRIEF HISTORY

1937
Isolation of the first-ever coronavirus, IBV, in chickens

1965
Coronaviruses are detected in humans

2002
The emergence of SARS

2003
The SARS epidemic is declared over

2005
SARS-CoV-1 is identified in horseshoe bats

2009
PREDICT project begins

2012
The emergence of MERS

2013
Found evidence of coronaviruses in bat guano harvested from caves in Thailand

2013
Identified 12 novel coronaviruses in bats from Mexico

2013
Detected two SARS-like novel coronaviruses in Chinese horseshoe bats

2013
Uncovered a wide range of coronaviruses circulating in bats from Saudi Arabia

2014
Provided evidence of undetected MERS in camels

2014
Recovered evidence supporting a role for camels in human MERS infections

2015
Discovered 6 new bat coronavirus host species in Thailand

2015
Informed IUCN guidelines to prevent respiratory disease transmission between humans and Great Apes

2016
Advocated for protection of bats and their ecosystems

2016
Published risk assessment of disease transmission in Lao PDR wildlife markets

2017
Led cross-sectional surveillance study of MERS-CoV in mammals of Egypt

2017
Characterized genetic diversity of bat coronaviruses in Southeast Asia

2017
Contributed further evidence for bats as the evolutionary source of MERS-CoV

2017
Published assessment of globl patterns in coronavirus diversity

2018
Found evidence of SARS-like coronavirus infection in humans

2018
Discovered fatal swine illness linked to novel coronavirus of bat origin

2018
Detected antibodies to MERS-CoV in dromedary camels in Bangladesh

2019
Published complete genome sequence of human coronavirus, HKU1

2019
Warned of potential for bat coronavirus spillover in rural Southern China

2020
Found high proportion of coronaviruses in rodents destined for human consumption in Viet Nam

2020
Investigated evolutionary history of ACE2 receptor use by SARS-like coronaviruses

2020
COVID-19 Pandemic declared by World Health Organization

2020
Detected coronaviruses in bats in Rwanda that are in close contact with humans

2020
Linked bat age and reproductive cycle to periods of coronavirus shedding

2020
Provided new evidence for assessing spillover risk in animal species

2020
Identified novel coronaviruses in cave-dwelling bats in Myanmar

2020
Detected coronavirus RNA in rodents in and around villages in Lao PDR

Prior to the emergence of SARS in 2002, coronaviruses were not thought of as having pandemic potential.

HUMAN CORONAVIRUSES HAVE WILDLIFE ORIGINS

Since the year 2000, there have been seven worldwide outbreaks of severe human disease with known or suspected animal origins (termed "zoonotic diseases") (NASEM, 2017). Three of those severe epidemics and pandemics were caused by viruses in the *Coronaviridae* family (coronaviruses; CoV). There are seven coronaviruses that are now known to infect humans; of these seven, four cause mild to moderate disease, symptoms associated with the "common cold," and gastrointestinal illness. The other three can cause severe respiratory illness and include SARS-CoV-1, the virus responsible for severe acute respiratory syndrome (SARS), MERS-CoV, the virus responsible for the much deadlier Middle East respiratory syndrome (MERS), and SARS-CoV-2, the devastating virus that causes coronavirus disease 2019 (COVID-19). What the coronaviruses responsible for SARS, MERS, and COVID-19 have in common is that they are all: 1) known or suspected to have evolved in a wild animal; 2) are capable of infecting multiple, diverse hosts; and 3) are suspected to have jumped into people from animals, likely as a result of human behavior, often passing through an "intermediate" species that has close contact with humans. Coronaviruses are suited to spillover into new species and adapt to new hosts; this characteristic is common in pathogens that can be very dangerous to public health.

HOW DOES A VIRUS RELATED TO ONES THAT CAUSE THE COMMON COLD GIVE RISE TO THE MOST SEVERE PANDEMIC IN THE LAST 100 YEARS?

Some answers can be found at the interface among wildlife, viruses, and people: it is here that the PREDICT project identified and characterized coronaviruses from around the world, searching for them in hotspots such as live animal markets, caves where bat guano is harvested, and communities that border wildlife habitats.

WHAT DID WE KNOW BEFORE PREDICT?

While the coronavirus responsible for COVID-19, SARS-CoV-2, was not identified until recently, coronaviruses have been known to the veterinary community since the 1930s (see timeline). In fact, the first human coronavirus infection was not identified until almost 30 years after coronaviruses were first discovered as disease-causing agents in animals. Prior to the emergence of SARS in 2002, coronaviruses were simply considered a nuisance to human health and were routinely found in domestic and wildlife species – they were certainly not thought of as agents of disease with pandemic potential. SARS awakened the world to the threat of an outbreak caused by a coronavirus and brought to light just how quickly a coronavirus can spread once it has made the jump into a naïve population, the effects of which would be felt for years after the epidemic was declared over. Our rapidly developing world offers opportunities for zoonotic diseases to quickly "go global", but zoonotic disease outbreaks have a way of catalyzing change and inspiring preventive action; to that end, the SARS pandemic increased the world's interest in viruses in wild animals and led to many studies looking to understand their potential to cause illness in people.

The emergence of SARS spurred a dramatic increase in information available on coronaviruses, as well as international collaboration for research. However, several years after the SARS pandemic ended, much about coronaviruses remained unclear, including which animal species can host coronaviruses and what can be done to reduce the risk of viral spillover. While building capacity to search for viruses in animals and people, the PREDICT project also wanted to address some of these unanswered questions.

Since 2009, PREDICT rapidly advanced the world's knowledge on the diversity of coronaviruses, prepared laboratories and field teams to be ready to respond to outbreaks, worked to identify risk factors for disease spillover that could be targeted for mitigation, and fostered community engagement and public education to reduce the potential for panic and confusion about emerging viruses.

WHAT DID PREDICT FIND?

Before PREDICT, the world's knowledge of coronaviruses was skewed towards known viruses with limited information about their circulation in animal hosts (especially bats in resource-limited countries). After collecting and testing samples from more than 88,000 animals and people from across 30 countries, PREDICT found 177 coronaviruses in animals and people, including 113 coronaviruses previously unknown to science (Table 1; Figure 1).

TABLE 1. *Number of coronaviruses detected by major host groups through PREDICT project surveillance (2009 – 2020).*

HOST GROUP	KNOWN CORONAVIRUSES	NOVEL CORONAVIRUSES
BATS	49	99
RODENTS / SHREWS	6	12
NON-HUMAN PRIMATES	1	1
HUMANS	4	0
DOMESTIC SPECIES	8	1
OTHER WILDLIFE	2	2
TOTAL	**64***	**113***

*Numbers do not total as some viruses were found in more than one host group

Domestic species includes cattle/buffalo, swine, poultry/other fowl, and dogs. Other wildlife includes duiker, birds (quail, ducks, geese), and civet.

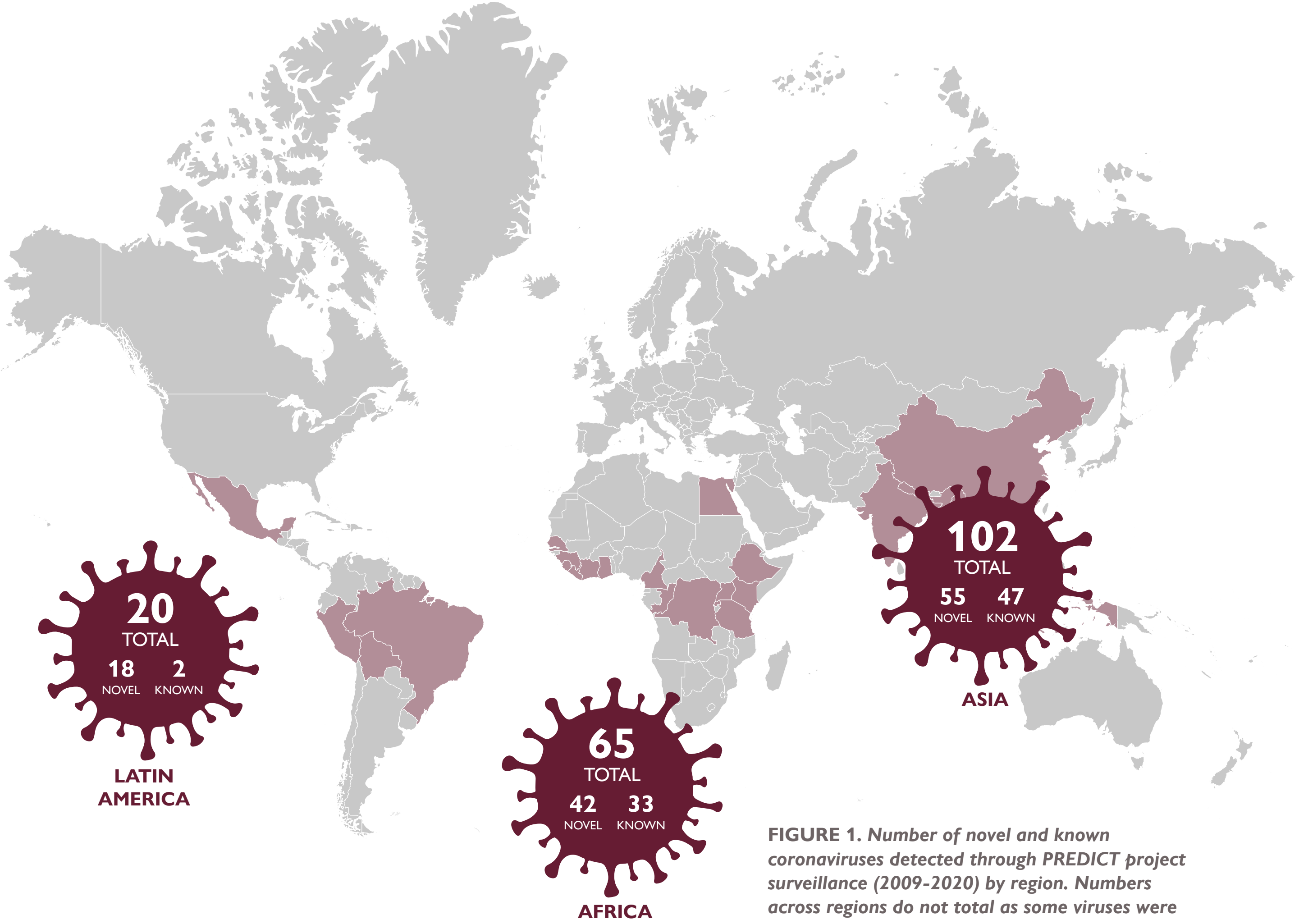


FIGURE 1. *Number of novel and known coronaviruses detected through PREDICT project surveillance (2009-2020) by region. Numbers across regions do not total as some viruses were detected in more than one region.*

PREDICT's work reinforced that bats, rodents, and primates are hosts for a number of viruses with zoonotic potential, including coronaviruses (Wacharapluesadee et al., 2015; Huong et al., 2020; Kreuder Johnson et al., 2020); but to understand which coronaviruses are likely to spillover into people, scientists need to do the foundational work in the lab and in the field to better understand the risky viruses' characteristics and their relationships with their animal host species. To address this knowledge gap, PREDICT explored the diversity of coronaviruses in more than 88,000 animals and humans in disease "hotspots" across Africa, Asia, and Latin America.

Not only did PREDICT estimate that over 3,000 coronaviruses exist in bats alone (many of which are undiscovered), we also found that bat biodiversity is directly correlated with coronavirus diversity (Anthony et al., 2017). Viruses found in bats belonged to all coronavirus groups, suggesting that certain species of bats are the evolutionary hosts (i.e. origin) of all these viruses. From PREDICT's wildlife sampling efforts, we also learned that a small number of coronaviruses have infected humans, despite there being thousands of different coronaviruses present among different species of bats. This finding suggests that most of these coronaviruses will not likely pose a large-scale threat to human health.

PHOTO: DIEGO MONTECINO-LATORRE

PREDICT also detected a high diversity of coronaviruses in animals destined for human consumption in Vietnam (Huong et al., 2020) and Lao PDR (Greatorex et al., 2016; McIver et al., 2020). These animals are part of the wildlife supply chain, or value chain, where wildlife are removed from their natural habitats and enter a vast trade network where they are transported, traded, and sold in overcrowded conditions to end consumers for consumption or for use in traditional medicine (see ***Special Feature: Forest to Table - The Wildlife Value Chain***). In Myanmar, PREDICT identified six new coronaviruses in bats, mostly in bat guano (i.e. bat feces) (Valitutto et al., 2020), and an analysis of guano samples from bats in Thailand supports the risk of coronavirus exposure to people when harvesting guano for use as fertilizer (Wacharapluesadee et al., 2013). In East Africa, PREDICT detected coronaviruses in bats that are in close contact with humans, such as in caves used for tourism (Nziza et al., 2020), and found that the reproductive cycles of East African bats correlates with periods of increased viral shedding, informing on timing for potential risk mitigation to reduce opportunities for coronavirus exposure (Montecino-Latorre et al., 2020).

PREDICT also made contributions to the race for effective treatments and vaccines for diseases caused by SARS-related coronaviruses. In 2015, PREDICT-identified viruses were tested for the ability to bind to human cells, and others found that some caused SARS-like illness in humanized mice (Ge et al., 2013; Menachery et al., 2015; Yang et al., 2015). Some of the SARS-related coronaviruses PREDICT identified were used by other scientists in studies testing the efficacy of promising antiviral drugs, such as Remdesivir (Baric et al., 2017). These adjacent studies demonstrated Remdesivir's potency against 'pre-pandemic' bat coronaviruses and helped in the rapid assessment of the only antiviral currently in use for COVID-19 patients, demonstrating the utility of finding viruses early and using them to prepare for pandemic threats.

THE CORONAVIRUS-HOST CONNECTION

Global trends in emerging viral threats confirm that spillover of viruses from wildlife to people is increasing (Jones et al., 2008). Biodiversity loss, climate change, land use change, human encroachment on wild spaces, and the wildlife value chain are forcing unnatural interactions among wildlife species, between wildlife and livestock, and, of course, wildlife and humans (Gibb et al., 2020), facilitating the spillover of new infectious pathogens. The way people interact with certain species of wildlife – particularly primates, rodents, and bats – put humans at risk for exposure to the viruses they carry, some of which have the potential to infect people.

The human immune system has defense mechanisms that combat infectious diseases, and most of the viruses with which we come into contact are eliminated by our immune defenses (Warren and Sawyer, 2019). Part of what makes new viruses problematic, including new coronaviruses such as the ones that cause SARS, MERS, and COVID-19, is that they are new to our immune systems. Our bodies have not seen these pathogens before and so we are slower to mount a response to infection. But what factors determine who or what is susceptible to coronavirus infection and disease? Research to understand how different viruses infect multiple hosts is ongoing, but we know that some coronaviruses have the ability to use human cell receptors for binding and cell entry. This ability is a critical first step for coronaviruses to be able to infect people.

For SARS-related coronaviruses, the key to host cell entry is via the angiotensin-converting enzyme 2 or ACE2 receptor. This protein is found on many different cell types throughout the body and is critical for regulating blood pressure and wound healing. Some SARS-related viruses are able to bind to the human ACE2 receptor (henceforth known as hACE2; Figure 2) which allows them to infect humans (Li et al., 2003). SARS-CoV-1 and SARS-CoV-2 are not closely related in their evolutionary history, but both viruses have the ability to use hACE2 to access and infect human cells (Li et al., 2003; Boni et al., 2020). SARS-CoV-1 and SARS-related viruses have been found in different species of horseshoe bats (family: Rhinolophidae), and therefore they are thought to be the ancestral hosts for many of the viruses in this group (Li et al., 2005; Ge et al., 2013; Hu et al., 2017). Animals that have ACE2 receptors may also be susceptible to some SARS-related coronaviruses. An understanding of which SARS-related viruses are and are not able to use the hACE2 receptor, and which animal hosts carry them, will be important for understanding the potential risk for spillover. While it seems unlikely that a random coronavirus should somehow jump from a bat to another susceptible species, disturbance of wildlife habitats by people and the contact therein introduces new pathways for disease spillover.

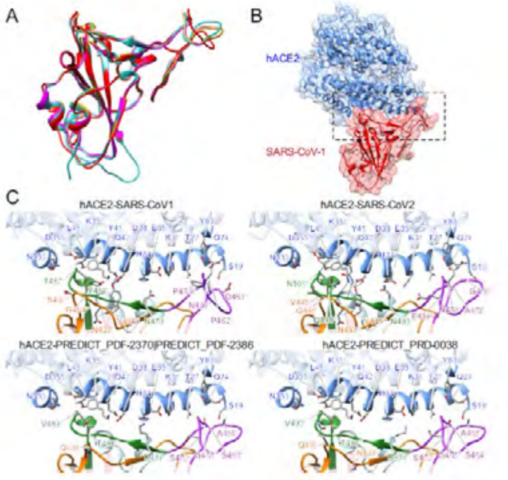


FIGURE 2. ***Structural modeling of sarbecovirus RBDs***

Experimentally, SARS-CoV-2 has been shown to bind to ACE2 receptors on cells from Chinese horseshoe bats (*Rhinolophus sinicus*), Malayan pangolins (*Manis javanica*), domestic cats, mink, and dogs. This indicates that these species have the potential to become infected, and indeed some have been infected through contact with SARS-CoV-2 positive individuals. PREDICT partners at the Wildlife Conservation Society reported that several tigers and lions were infected with SARS-CoV-2, having been likely exposed from an asymptomatic staff person who cared for them, marking the first known case of SARS-CoV-2 transmission from a person to a wild animal (Wildlife Conservation Society, 2020).

PHOTO: GORILLA DOCTORS

CORONAVIRUS CARRIERS: BATS

Bats and their viruses are continually coevolving in what is referred to as an evolutionary arms race.

Given currently available data, most scientists agree that SARS-CoV-1 and potentially other SARS-related coronaviruses are most likely of bat origin (Boni et al., 2020; Wells et al., 2020). To that end, PREDICT also identified a number of SARS-related viruses in bats, as well as insights into the evolutionary history of these viruses and the ecology of some bat hosts (see timeline). Despite their integral, ecological contributions to seed dispersal, pollination, and pest control (Kunz et al., 2011), bats are generalized and mischaracterized as inherently dangerous to human health in part due to the concern of the number of pathogens they (as an animal group) can carry.

Even though bats can be found in nearly every ecosystem in the world, we are just now piecing together their natural history and understanding behaviors that shape the relationship between pathogens and certain bat species. Some bats have very long lifespans, and many are highly social, often living in colonies that can range up to millions of animals (Voigt and Kingston, 2016). Large populations, where bats are in close contact with one another, are ideal settings for the transmission of viruses among individuals. Bats are also particularly sensitive to environmental disturbance, and research suggests habitat disruption by humans (as well as other environmental threats) may cause chronic stress in forest-dwelling bats (Jones et al., 2009; Seltmann et al., 2017), impairing normal immune system function and potentially creating opportunities for coronaviruses (and other pathogens) to move from species to species.

With at least 1,400 species, bats are one of the most abundant and diverse animal groups on the planet. Given the remarkable diversity of bat species and the physiological and behavioral variability among them, there are likely a multitude of traits among different species that impart unique "antiviral" abilities to bats (Zhang et al., 2013; Banerjee et al., 2020; Brook et al., 2020). As with other hosts, bats and their viruses are continually coevolving in what is referred to as an "evolutionary arms race", where a virus must continuously evolve and create new versions of itself to evade hosts' immune defenses. In turn, bats evolve mechanisms to endure persistent viral infections, such as immune system defenses that force viruses to rapidly adapt or die. Some versions of the virus will be thwarted by the bat immune system; others will survive at low-levels in the host (Plowright et al., 2016; Banerjee et al., 2020) or "disappear" locally while persisting at the population level, after being passed on to other bats.

Unique to bats is their ability, as a mammal, to maintain sustained flight; some researchers believe this extraordinary ability has shaped the immune system of some bat species, imparting "protective" immune defenses against otherwise pathogenic viruses (Banerjee et al., 2020). Maintaining flight is hard on a body and uses large amounts of energy, causing body temperatures to skyrocket and an overproduction of destructive molecules, known as "metabolic waste". Waste can damage host DNA, so bats have naturally evolved mechanisms to maintain high body temperatures and manage waste byproducts. These adaptations appear to also allow them to ward off diseases. For example, some bats have a "dampened" immune response that provides relief from or entirely avoids inflammation. For many diseases, such as COVID-19, we are learning that an overactive immune response exacerbates disease pathology in some people. Studying how the bat immune system responds to viral infections may lead to better therapeutics and vaccines for humans.

CORONAVIRUS SPILLOVER POTENTIAL: A HIGH-RISK PATHOGEN

HEALTH CONCERNS FROM THE WILDLIFE VALUE CHAIN

Across the globe, the wildlife value chain facilitates the trade, slaughter, and consumption of animals, ranging from pangolins (the most trafficked animal in the world) to small reptiles and waterfowl. During the SARS epidemic in 2002-2003, an international collaborative research effort sampled animals at wildlife markets across the Pearl River Delta region in China and found several species of wildlife carrying SARS-related coronaviruses, including but not limited to a raccoon dog (*Nyctereutes procyonoides*), Chinese ferret badgers (*Melogale moschata*), and Himalayan masked palm civets (*Paguma larvata*), the latter of which were suspected to be an intermediate host for transmission of SARS-CoV-1 to humans (Guan et al., 2003). Further support for masked palm civets as a source of infection came when multiple epidemiological studies reported that some SARS-positive patients were in contact with civets, including a waitress at a restaurant that housed live palm civets being served as exotic cuisine. These civets were reportedly purchased in a local live animal market (Song et al., 2005; Wang et al., 2005).

Given the many opportunities in the live market trade network for wildlife to come into contact with humans, incidentally or intentionally, PREDICT has investigated the potential role of wildlife species, including bats and rodents, to host coronaviruses in live animal markets. Field teams in Lao PDR observed seven high-volume, live animal markets and collected information about the species of wildlife traded, market prices, biosafety and biosecurity in the markets (protocols and procedures meant to protect human and animal health from disease), and the origins of patrons. PREDICT found that, not only was the rapid turnover of wildlife frequent in these markets, but highly diverse animal groups were represented at the market (Greatorex et al., 2016). Among those observed in large numbers were rats (family: Muridae), fruit bats (family: Pteropodidae), and civets (family: Viverridae). Experimental infections have shown that fruit bats and civets are capable of potentially hosting and shedding coronaviruses under the right conditions (Wu et al., 2005; Schlottau et al., 2020).

PREDICT reported further evidence of viral shedding along wildlife supply chains as a result of coronavirus surveillance in Viet Nam at high-risk interfaces for disease transmission (Huong et al., 2020). Rats are a common source of food in Viet Nam, where they are commercially farmed or wild-caught and transported live to markets and restaurants via the "field-to-fork" value chain. We found that farmed rats destined for trade and human consumption harbored multiple known coronaviruses, with the proportion of positive samples increasing as animals moved up the supply chain from their initial trapping towards consumption (see ***Special Feature: Forest to Table - The Wildlife Value Chain***). The triple threat of increasing prevalence of coronaviruses in wildlife destined for market sale, commingling of wildlife in crowded and stressful transport conditions along the supply chain (with the potential for virus sharing), and chances for wildlife-human interaction give insights into how zoonotic pathogens make the jump from one species to another.

Research around the animal origins and emergence of SARS-CoV-2 is ongoing, but some evidence suggests that a spillover event could involve live animal markets. It is also proposed that one or more infected persons spread the virus at the market, without the virus originating in the market itself (known as a "superspreader" event). Given the prevalence of coronaviruses in wildlife, many have called for an immediate blanket-ban on the global wildlife trade. These calls quickly dominated conversations about pandemic prevention; however, there is due concern that sweeping laws solely targeting the wildlife trade would not only have significant consequences for sustainable development initiatives, but potentially ignore other significant drivers of disease emergence, such as land-use change and deforestation (Lindsey et al., 2020). While there is an urgent need to address wildlife trade practices that are unsustainable and threaten human and animal health, especially those that are illegal, we must recognize that the wildlife is also an important food resource for many communities, and some vulnerable groups rely on hunting wild animals as their main source of protein (Wildlife Conservation Society, 2020; Roe et al., 2020). Sustenance hunting differs from the commercialization of wild animal meat and byproducts, and through PREDICT's work in Southeast Asia, we know that the risk of emergence and transmission of coronaviruses amplifies as animals move through the commercial supply chain (Huong et al., 2020).

Results from our work supports recommendations to reduce risk for spillover and spread of coronaviruses via the wildlife value chain through stricter biosafety controls and better enforcement, as well as minimizing effects on the livelihoods of local communities with risk-reducing education.

IMPACTS ON GLOBAL AGRICULTURE & FOOD SECURITY

Coronaviruses have also been linked to domestic animals, including livestock, that are a conceivable threat to the agricultural sector. In 2012, cases of MERS were traced to individuals with self-reported contact with livestock and/or farm animals, including ownership or occupational contact with dromedary camels, which led to suspicions of inter-species transmission. Investigators concluded that MERS-CoV must have an animal host and that it was repeatedly spilling over from animals to people, perpetuating the spread of the virus (Breban et al., 2013).

Scientific consensus now supports that dromedary camels are an intermediate animal host for MERS-CoV transmission to people, and data also suggest that insectivorous bats are the evolutionary host for the virus (Memish et al., 2013). A collaborative team led by researchers from Saudi Arabia, and including PREDICT scientists, conducted a thorough survey of dromedary camels in Saudi Arabia and found evidence suggesting that a MERS-like virus or a related virus had been circulating in camel populations undetected for decades before the first known human case of MERS in the summer of 2012 (Alagaili et al., 2014).

China is both the largest producer and largest consumer of pigs worldwide. As such, the spread of highly contagious, lethal disease among pigs is a constant threat to human food systems and animal health, with the potential for catastrophic effects to global food security. In October 2016, a series of mysterious pig die-offs impacted four separate commercial pig farms in southern China, leading to the death of nearly 25,000 piglets. A multidisciplinary team of researchers, including PREDICT scientists, discovered a novel coronavirus now known as swine acute diarrhea syndrome coronavirus, henceforth SADS-CoV (Zhou et al., 2018). SADS-CoV is closely related to SARS-CoV-1. Interestingly, this pig virus belongs to the same group as SARS-CoV-1 and SARS-CoV-2, revealing that some of these viruses can affect animal health. PREDICT experimentally tested the ability of SADS-CoV to infect other species. Evidence to date shows that SADS-CoV does not appear to be capable of infecting people; nonetheless, the rapid spread of a coronavirus that causes severe and fatal disease in livestock and poultry could have massive ramifications for the human global food system and a huge impact on meat demand both locally and abroad (Karesh et al., 2012).

PHOTO: SMITHSONIAN INSTITUTION

CONSIDERATIONS FOR CONSERVATION EFFORTS

Given that great apes have a close genetic relationship to humans and that clinical manifestations of many infectious diseases present similarly in great apes and humans (Calvignac-Spencer et al., 2012), great ape populations are especially threatened by coronavirus transmission. Historically, human respiratory pathogens have been associated with illness and sometimes death in wild gorillas, including severe human metapneumovirus (HMPV) infections (Palacios et al., 2011) and human respiratory syncytial virus (HRSV) infections (Grützmacher et al., 2016). Both HMPV and HRSV can cause asymptomatic infections, and while there is no evidence to date, it is possible that SARS-CoV-2 and MERS-CoV could have similar clinical signs in great apes (Hall et al., 2001; Al-Tawfiq and Gautret, 2019; Oran and Topol, 2020). Further, human respiratory pathogens have been documented to simultaneously infect gorillas and local human populations (Grützmacher et al., 2016), and a coronavirus reportedly spread from humans to chimpanzees in Tai National Park, Côte d'Ivoire, causing an outbreak of mild respiratory disease (Patrono et al., 2018), underscoring the need for disease prevention and control measures to avoid pathogen transmission.

PREDICT works in partnership with Gorilla Doctors and the Mountain Gorilla Veterinary Project (MGVP) to actively survey for pathogens that are a danger to human and gorilla health in Uganda, Rwanda, and eastern Democratic Republic of the Congo (DRC). In 2012 and 2013, outbreaks of respiratory disease occurred in endangered mountain gorillas (*Gorilla beringei beringei*) in Virunga Massif, Rwanda. The PREDICT team collected and tested samples from clinically ill mountain gorillas during two separate outbreaks and detected a strain of HRSV closely related to HRSV strains that circulate in humans (Mazet et al., 2020). Collective findings to date suggest repeat introductions of human respiratory viruses, such as HRSV, are an ever-present threat to gorilla conservation and that additional biosafety protocols must be strictly enforced in wildlife tourism and observational research that puts humans in close proximity to wildlife (Mazet et al., 2020; Gilardi et al., 2015).

PHOTO: GORILLA DOCTORS

At present, there is no evidence to suggest that SARS-CoV-2 has or can infect wild gorilla populations; however, gorilla tourism in Africa was suspended for several months to protect the health and safety of tourists, the gorillas, and their protectors. Despite mounting evidence of human-to-gorilla pathogen transmission, the revenue generated from ecotourism helps governments continue to protect wildlife habitats, and pandemic-related economic fallout will likely reduce availability of funds for conservation-related efforts (Lindsey et al., 2020). The ecotourism industry that exists to support endangered wildlife, ecosystems, and the communities that depend on them faces complex challenges in the protection of threatened wildlife and ecosystems from coronaviruses.

IMPROVE SYSTEMS TO STOP SPILLOVER, AMPLIFICATION, & SPREAD

DETECTING CORONAVIRUSES EARLY & AT THEIR SOURCE

Cases of disease often go unreported to public health systems until there is a major incident, especially in regions of the world where healthcare facilities are hard to come by or treatment is expensive to pursue. Thus, small outbreaks of disease or isolated cases of infection are often overlooked, despite possibly being caused by harmful pathogens of serious concern. Detection and control in the early-phases of disease outbreaks are critical to containing the spread of disease; to meet this need, PREDICT partnered with local hospitals and health clinics to identify communities that were most at-risk, living near or in disease hotspots (Olival et al., 2017) and conducted cost-efficient, targeted disease surveillance in these locations that led to valuable insights on coronavirus distribution.

Over the life of the PREDICT project, we have experimentally identified SARS-related coronaviruses that can bind to human cells and showed that people living and working at the wildlife-human interface in rural China were exposed to coronaviruses (Wang et al., 2018; Li et al., 2019). In Africa, PREDICT assessed risks involved with human-bat contact and identified seasonal trends in coronavirus shedding (Montecino-Latorre et al., 2020; Nziza et al., 2020) – these findings can guide policy aimed at the prevention of spillover and provide ethical alternatives to reducing bat-human conflict. Around the world, work funded by PREDICT has contributed to large libraries of virus sequences and modeled a professional culture of scientific integrity and global data sharing that allowed scientists to quickly classify the SARS-CoV-2 virus and begin investigating its origins.

EQUITABLE APPROACHES TO REDUCING PANDEMIC RISK POSED BY CORONAVIRUSES

Vaccines and better treatments are needed to combat illnesses caused by coronaviruses, but creating drugs and therapeutics to combat every coronavirus that crops up is an expensive, unsustainable endeavor. Avoiding activities and behaviors that amplify the risk of disease is both cost-efficient and highly effective in preventing pandemics (Dobson et al., 2020). To this end, not only did PREDICT investigate the potential spillover of viruses into people at high-risk interfaces but it also engaged with local communities and carried out educational programs, which served to both inform the public about activities that increase zoonotic risk and suggest behavioral modifications to make these activities safer for people and animals. Modifications and interventions ranged from promotion of correct personal protective equipment and hygienic practices when handling animals to closing high-risk bat cave tourism sites and implementing large-scale education and awareness campaigns, such as the *Living Safely with Bats* risk reduction and behavior change communication intervention initiated in Sierra Leone and taken to scale in all PREDICT project countries. (see *Special Feature: Living Safely with Bats*).

BUILDING A ONE HEALTH WORKFORCE

To assist policy makers with pandemic preparedness and response, the international scientific community must collaborate across the human, animal, and environmental health sectors. One of PREDICT's greatest achievements towards a world better prepared to confront pandemic threats has been the creation of a multidisciplinary global workforce composed of One Health professionals in scientific institutions, national laboratories, and government ministries. At the onset of the COVID-19 pandemic, PREDICT-trained, in-country laboratory researchers and field teams rapidly detected and isolated the virus in their home countries – a testament to PREDICT's efforts in building national capacity for virus diagnostics – and evidence that supporting disease surveillance and laboratory diagnostic systems, specifically those in low and middle income countries, is crucial to global health security (see *Chapter 6* and *Chapter 7*).

INVESTING IN PREPAREDNESS

As our world becomes more globalized, pandemic threats should be a global priority. As we have seen with the COVID-19 pandemic, once an outbreak of disease is underway there is a short window of time to learn about the pathogen and develop a response plan. International partnerships, supported by programs like the Global Health Security Agenda, promote international trust and avenues for communication between scientists and policy makers. Investments in preparedness allowed the PREDICT project to prepare laboratories and countries to be able to react and effectively respond to a coronavirus threat in real-time.

The World Health Organization maintains a list of priority zoonotic diseases that are in need of attention from the global health community due to their international epidemic potential. In 2018, 'Disease X' (caused by 'Pathogen X', an unknown agent of human disease) was added to this shortlist (WHO, 2020). Disease X could have been any type of pathogen (bacteria, virus, parasite, fungi, or prion), but evidence from the PREDICT project and the greater scientific community suggested zoonotic RNA viruses, like SARS-CoV-2 and other coronaviruses, were poised to trigger the next pandemic (Woolhouse et al., 2008; Kreuder Johnson et al. ,2015), underscoring the need for continued funding for large-scale pathogen discovery and surveillance programs (Carroll et al., 2018; Daszak et al., 2020). If the world continues to react and respond inefficiently to large-scale disease outbreaks and support continues to follow pandemics instead of preceding them, we will always be playing an expensive game of catch-up with the next emerging disease threat.

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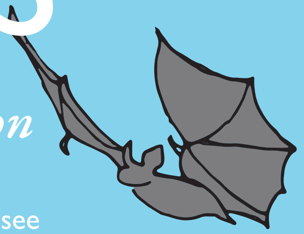
SPECIAL FEATURE

LIVING SAFELY WITH BATS

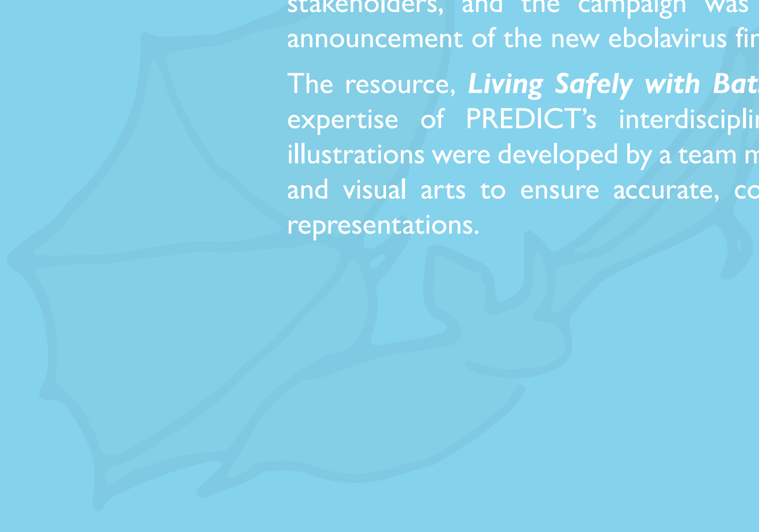


Innovative Risk Reduction Intervention

Following the discovery of a new ebolavirus in bats in Sierra Leone (see *Special Feature: Ebola Host Project*), PREDICT worked closely with USAID and local partners to rapidly develop a communications plan centered on a risk reduction and behavior change communication intervention strategy. Our goal was to help raise awareness at the community level about ways to reduce disease risks associated with human-to-bat contact.

We worked with the PREDICT project's One Health team to identify the most culturally appropriate, feasible, and effective intervention style, and we developed a framework for assessing potential materials, channels of communications, respective audiences, and core messaging. A moderated picture book, delivered by a trusted community leader, was selected as the best tool to put into the hands of our local teams and in-country stakeholders. We also developed a communications plan to ensure a well-coordinated effort with government and community stakeholders, and the campaign was synchronized with the public announcement of the new ebolavirus finding.

The resource, ***Living Safely with Bats***, leveraged the subject matter expertise of PREDICT's interdisciplinary One Health team. The illustrations were developed by a team member trained in animal biology and visual arts to ensure accurate, consistent, and compelling visual representations.




Exemple:
Devoir:
Correction de devoir!

To vet and refine the resource, we held focus groups with project subject matter experts and we solicited feedback and cultural input from **17 PREDICT country teams** (Bangladesh, Cambodia, Cameroon, Cote d'Ivoire, Democratic Republic of the Congo, Ghana, Guinea, Indonesia, Lao PDR, Malaysia, Nepal, Republic of Congo, Senegal, Sierra Leone, Tanzania, Thailand, and Viet Nam).

The ***Living Safely with Bats*** resource was piloted in Sierra Leone and Tanzania (in collaboration with a US Department of Defense, Defense Threat Reduction Agency project also working at the bat-human interface) to garner input on ways to improve both the content and the process of delivery. A comprehensive review of the resource was also conducted in Guinea. The PREDICT project's behavioral science experts were present in West Africa to train and support country partners during the launch of the resource, and beginning in July 2018, it was successfully delivered to community partners during outreach events in Sierra Leone, Guinea, and Liberia.

Section 1. Bats are an Essential Part of our Ecosystem

Bats play an important role in keeping us and our ecosystem healthy. For example, bats spread seeds from hardwood and fruit trees.

Africa - July 2018 version

FIGURE 1. ***Illustrations with moderator talking points.***

FIGURE 2. ***PREDICT collaborated with local partners to raise community awareness on ways to reduce disease risks associated with human-to-bat contact.***


Living Safely with Bats


Vivre en en toute sécurité avec les chauves-souris


ከሌሊት ወፎች ጋር ካለችግር መኖር


ការរស់នៅដោយសុវត្ថិភាពជាមួយសត្វប្រចៀវ


လင်းနို့များနှင့်အတူ အန္တရာယ်ကင်းစွာ နေထိုင်ခြင်း


चमेरोसँग सुरक्षित रहने


An toàn chung sống cùng loài Dơi


Hidup Bersama Kelawar Secara Selamat


Kuishi salama na popo


ການດຳລົງຊີວິດຢູ່ຮ່ວມກັບໄຕເຈຍຢ່າງປອດໄພ


Hidup Aman Berdampingan dengan Kelelawar


อยู่ร่วมกับค้างคาวอย่างปลอดภัย

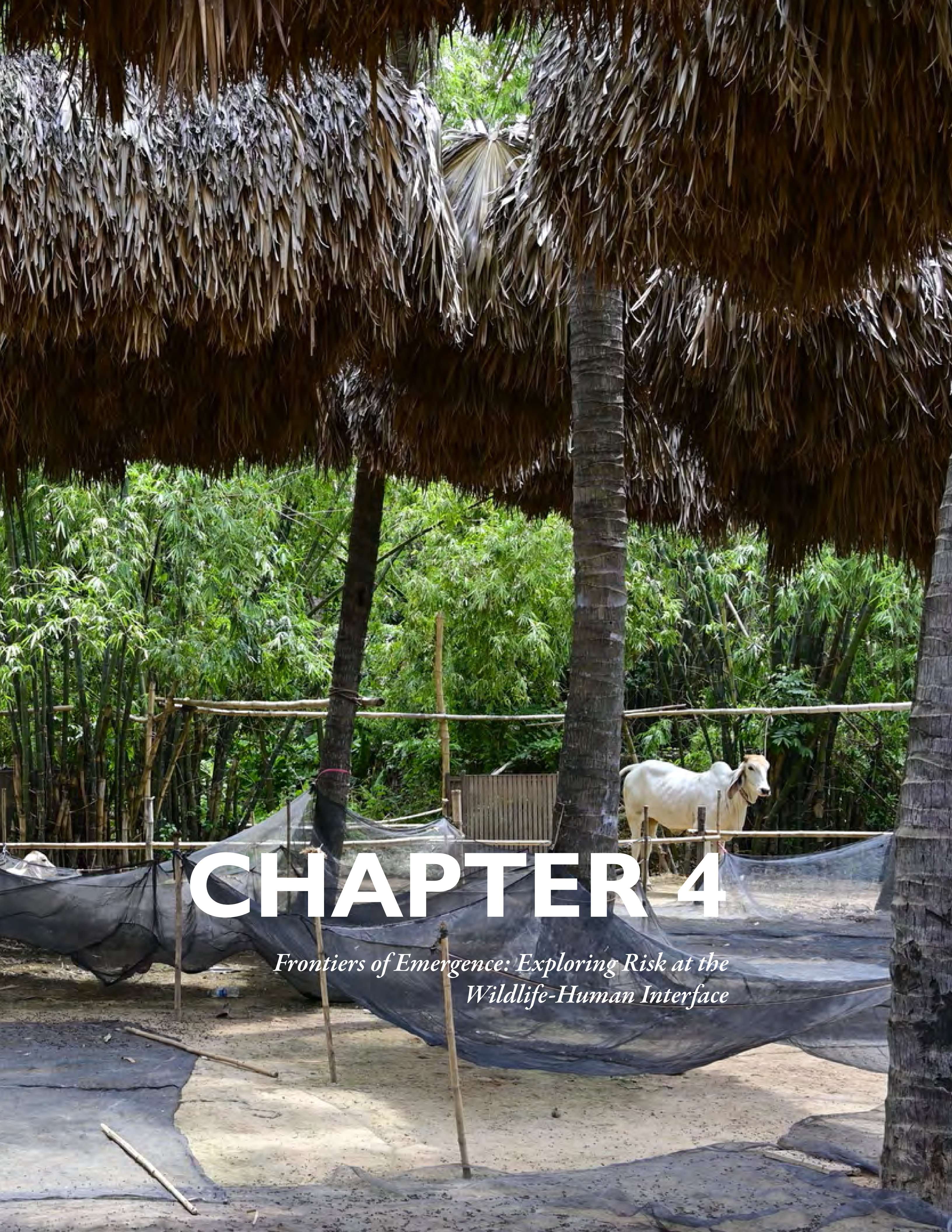
Since the initial development of the resource for Africa, ***Living Safely with Bats*** has been adapted to Asian contexts, with content developed on bat hunting, guano harvesting, and cave-related tourism, as these high-risk bat-human interfaces are specific to countries in that region. This resource was translated into 12 languages and has been broadly distributed at national and community levels throughout Africa, Asia, and beyond.

The ***Living Safely with Bats*** risk reduction and behavior change communication resource is available at:

p2.predict.global/living-safely-with-bats-book

> ***"Things were easier thanks to the bat book and the communication skill of the team members. The bat book is an excellent tool, especially for kids who understand the pictures and the messages."***
>
> –Dr. Amaoudand Kalivogui "Kali"
> PREDICT Guinea - EHP

PHOTO: PREDICT CAMBODIA



CHAPTER 4

Frontiers of Emergence: Exploring Risk at the Wildlife-Human Interface

KEY TAKEAWAYS

- With our teams around the world, we discovered 958 novel viruses in wildlife. The highest numbers of different viruses were detected in animals that were hunted and those sampled near people's homes, and sampled in and around their farms and fields.
- Viruses detected in our four high priority groups (coronaviruses, flaviviruses, paramyxoviruses, and influenza viruses) were more likely to be detected from animals sampled during the wet season, compared to the dry season, critical information for improving the probability of detection and cost effectiveness of surveillance efforts.
- We developed machine learning models that help identify viruses with the potential to infect people and found that the coronaviruses we detected have the ability to infect a greater number of animal species than viruses from other groups.
- Our data show that bats, followed by rodents and shrews, are at higher risk for shedding viruses belonging to these priority groups, including coronaviruses, compared to other wild mammals. Further investments are needed to better understand the virologic, ecologic, and epidemiologic features that put people in contact and at-risk from bat-borne coronaviruses, especially specific genetic features that could inform on their potential to infect people.
- We identified several animal-human interfaces with appropriate conditions for viral spillover to occur, along with control points for intervention. Wildlife value chains, including markets, are critical control points for future spillover and present opportunities for risk mitigation through targeted and evidence-informed behavior change communication approaches.
- Our investigations using serological techniques to reveal previous pathogen exposure provided evidence for under-reported and unrecognized spillover events that are likely more common than we realize, influencing priorities for in-depth future investigations at high-risk interfaces.

Before dawn, wearing a raincoat, gloves, and straw hat, several women and children hike through the forest to the cave entrance and move into the cavern. By flashlight, some wearing scarves or applying balm to their faces to deal with the smell, they work under a living canopy, bats now returning to roost. From the cave walls and floor, they scrape guano into a woven sieve, then into bags, neatly lined up on the cave floor. These bags will be carried to traders and then sold to farmers as rich fertilizer for crops and orchards. We know that the bats in these caves and their guano is not only rich in nutrients but in potentially infectious viruses. This cave is a high-risk wildlife-human interface, where viruses circulate in their natural reservoir, capable of leaping from nature into people because of our behaviors.

EVALUATING SPILLOVER RISK: OUR APPROACH

PHOTO: SMITHSONIAN INSTITUTION

Our team explored the risks of viral spillover at this interface and others that have a high potential for zoonotic pathogen exposure. Across the PREDICT project, we have found that we can learn a great deal about risk by finding which wildlife are shedding potentially zoonotic viruses and where. In doing so, we have assessed which wildlife-human interfaces may be highest risk for spillover, and now we better understand who is at risk and why. These insights provide an evidence base for interventions and strategies to prevent spillover and to keep us safe. Our findings also inform future surveillance efforts that will use a broad-based investigative approach to not only identify known viral threats, but to also detect novel and emerging viral threats, the known unknown.

We identified high-risk wild animal-human disease transmission interfaces, at which zoonotic viral transmission from animals to humans occurred most frequently, based on a rigorous and detailed examination of published peer-review papers. Investigation into the mechanisms of transmission of all zoonotic viruses from animals to people revealed that viral transmission happens primarily at high-risk interfaces, including in and around human dwellings, in agricultural fields, and in occupations, such hunting as well as working in research, wildlife management, and at zoos or wildlife sanctuaries (Kreuder Johnson et al., 2015, Figure 1). Primate hosts were most frequently cited as the source of viruses transmitted by direct contact during hunting, while rodent hosts were more likely to be implicated in transmission by indirect contact in and around human dwellings and in agricultural fields. These findings informed high-risk interfaces that would be the focus of surveillance in the later phases of the PREDICT project. The world’s knowledge of zoonotic viral transmission at high-risk interfaces is incredibly relevant baseline knowledge that we used to communicate risk to partners, inform disease mitigation strategies, and place PREDICT findings into context.

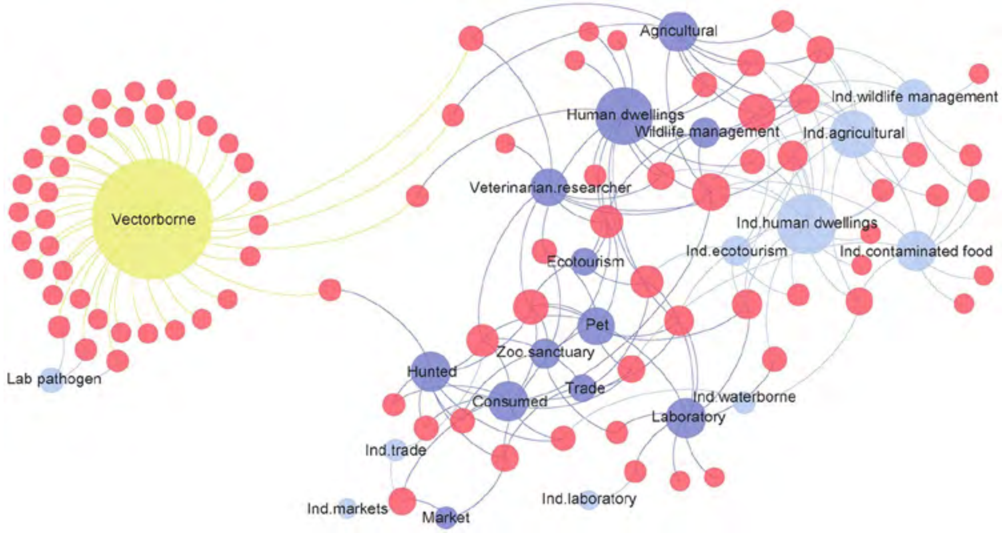


FIGURE 1. ***Two-part network showing high-risk disease transmission interfaces shared by zoonotic viruses transmitted from wildlife to humans. Blue and yellow circle sizes for high-risk interfaces are proportional to the number of viruses reported for each transmission interface. Colors refer to direct contact with wildlife (dark blue), indirect contact with wildlife (light blue), and vector transmission (yellow). Red circle size, each representing one of 86 viruses reflects the number of connections to different transmission interfaces and the ecological plasticity of viruses through the use of multiple transmission opportunities. Highly connected and more central interfaces facilitated transmission of more viruses, providing an epidemiologic picture of circumstances likely to promote future disease emergence and important targets for disease surveillance and preventive measures (Johnson et al., 2015).***

PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

PREDICT implemented field surveillance to identify viruses at high-risk interfaces, particularly prioritized viruses in the coronavirus, paramyxovirus, flavivirus, and influenza virus groups. We also evaluated the frequency of virus detection at these interfaces to provide insight into epidemiologic situations that are most likely to facilitate zoonotic transmission. Comparison of risk at interfaces was relative to the frequency of virus detections across all four virus groups for specimens collected from wild mammals from 2014-2019.

WHERE WILL VIRUSES SPILL OVER?

PREDICT focused on concurrent wild animal and human surveillance at wildlife-human interfaces that included caves, live animal markets, agricultural settings, and natural areas undergoing rapid transitions in land conversion and anthropogenic change (Figure 2; see ***Special Feature: What is the Wildlife-Human Interface?***). The majority of wildlife sampling occurred around human dwellings, a high-risk interface where One Health surveillance using human and animal data collection could be optimized. Wildlife in crops and fields (raiding crops or near people working their crops or grazing animals), hunted wildlife, wildlife in live animal markets or the animal supply chain, wildlife near ecotourism sites such as caves, and wildlife destined for consumption were also frequently sampled (Figure 2).

WHAT WE FOUND

Unique viruses from the four targeted virus groups were frequently detected in animals sampled at the high-risk interfaces listed in Table 1. The highest number of individual viruses were detected in animals sampled in human dwellings, near crop production, and in animals that were hunted. Although less than 3,000 bats were sampled in caves (Figure 2), a relatively high number of different viruses across several virus groups were detected in bats at the cave interface, emphasizing that bats in cave habitats can sustain a high diversity of viruses.

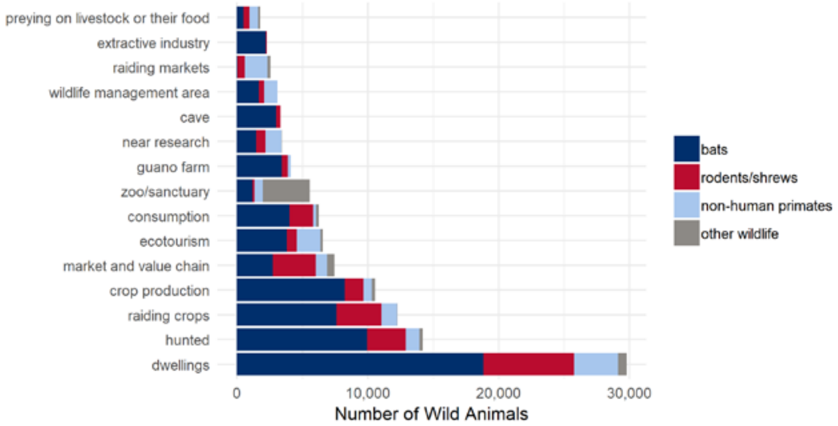


FIGURE 2. ***Number of wildlife sampled and tested at high-risk interfaces from Oct. 2014 to Sept. 2019 in Asia and Africa. More than one disease transmission interface was identified for many animals. Includes all interfaces at which at least 100 animals were sampled.***

INTERFACE	**# UNIQUE VIRUSES DETECTED**
Dwellings	122
Crop production sites	94
Hunted	84
Ecotourism sites	55
Raiding crops	50
Consumption	45
Public safety hazard (threat of interactions with people)	30
Wild animal sanctuaries	28
Extractive industry sites	24
For sale in large market (>20 vendors)	23
Caves	18
Guano farms	17
Private sale	17
In transit along value chain	15
For sale in medium market (5-20 vendors)	13
Wildlife management area	12
For sale in small market (<5 vendors)	12
Near research activities	11

TABLE 1. ***High-risk interfaces at which more than 10 unique viruses from the four targeted virus groups were detected in animals across all sample sites by PREDICT teams (2014-2019). Numbers in this table do not reflect frequency of detection, only numbers of different viruses.***

Viruses identified by PREDICT teams were significantly more likely to be detected in bats, followed by rodents and shrews, compared to other mammals.

Epidemiologic risk models were developed to assess the probability of a positive virus test result for each specimen tested, based on risk factors related to mammalian taxonomic class, animal condition at sampling, specimen type, sampling season, and disease transmission interface. Our modeling framework adjusted for relative sampling effort and differential detection among laboratories in order to summarize general patterns for many viruses within multiple virus groups.

Viruses identified by PREDICT teams were significantly more likely to be detected in bats, followed by rodents and shrews, compared to other mammals. Overall, 9.7% of bats were positive for coronaviruses, paramyxoviruses, flaviviruses, or influenza viruses, compared to 4.7% for rodents and shrews. Specimens from all wild mammals were more likely to be detected with prioritized viruses in the wet season compared to the dry season. Among all specimen types tested, we found that guano, urogenital swabs, and urine specimens were the most likely to be positive for prioritized viruses, likely reflecting the natural history for the viruses we targeted. Rectal swabs, followed by feces, and then oral swabs were less likely to be positive, but still significantly more likely to be positive than blood and tissue specimens. Building on findings from the first five years of PREDICT data (see *Chapter 1*), the PREDICT strategy focused on sampling live animals or freshly dead animals that were observed to be in good condition to avoid animals that were decomposed and unlikely to test positive for RNA viruses. We did not detect a difference in virus detection between sampled live animals and freshly dead animals.


PHOTO: PREDICT CôTE D'IVOIRE

We also constructed specific models for coronaviruses and paramyxoviruses (the family to which the deadly Nipah and Hendra viruses belong). Insights from modeling revealed that specimens from bats were approximately three times more likely to test positive for coronaviruses than specimens from rodents and shrews, with guano being the highest risk specimen type. Similarly, bats were twice as likely to be positive for paramyxoviruses compared to other mammals, and these viruses were most commonly detected in urine and urogenital swabs.

HEIGHTENED VIRUS DETECTION IN THE PERFECT SETTING FOR SPILLOVER

Risk at each animal-human interface was compared based on the frequency of each virus detected and then summed across all identified coronaviruses, paramyxoviruses, flaviviruses, and influenza viruses. Among all interfaces explored, we found that wild mammals sampled while in transit along the value chain were the most likely to test positive, followed by wild mammals sold at markets (see our ***Special Feature: Forest to Table - The Wildlife Value Chain***). The risk of the wildlife value chain and markets identified for the four virus groups held true for coronaviruses and paramyxoviruses when evaluated on their own, with intensified risk also identified in caves and sanctuaries for coronaviruses and wild animal farms for paramyxoviruses.

The wildlife market and value chain are, in many ways, the perfect epidemiological settings for virus evolution and adaptation towards zoonotic potential. Risk of viral infection in the wildlife value chain is amplified by the frequently dense housing conditions, often with multiple and wide-ranging species housed together. Unfortunately, these wild animals are also in close contact with livestock and people in these settings, providing opportunities for spillover. Our data provide evidence that the wildlife value chain, including markets, are critical points for controlling future spillover opportunities. People and animals in these high-risk settings are an important focus for ongoing surveillance and should be targeted for collaborative mitigation activities to improve biosecurity and reduce risk.

PHOTO: PREDICT VIET NAM

INTENSIFIED RISKS IN THE WILDLIFE SUPPLY CHAIN

Livelihoods and professions in the wild animal value chain include hunting and capture of wildlife, transport of wildlife, and ultimately sale of animals (ranging from private sales, to small, medium, and large markets, to restaurants). Sites along the supply chain for wildlife sold at markets were visited by PREDICT teams between July 2015 and November 2018, with the goal of visiting each site at least once during the wet and dry season of each year. These included 562 data collection events across 61 sites in 13 African/Middle Eastern countries (Cameroon, Democratic Republic of the Congo, Egypt, Ethiopia, Ghana, Guinea, Côte d'Ivoire, Jordan, Kenya, Republic of the Congo, Sierra Leone, Tanzania, Uganda) and 65 sites in 8 Asian countries (Bangladesh, Cambodia, Indonesia, Lao People's Democratic Republic, Myanmar, Nepal, Thailand, Vietnam). During each visit, observers recorded the node along the supply chain (whether the site was the source of animals to be sold or at the point of sale), whether wild and domestic animals were present (alive or slaughtered on site, dead or sold in parts), whether animals were housed together, and if veterinary care and biosecurity protocols were available.

Of all sites characterized, 45% (n=57/126) were found to be selling live wildlife or were slaughtering wildlife on site. More sites in Asia were also selling domestic animals alive or slaughtered on site than sites in Africa (91% of sites in Asia, 66% of sites in Africa). While multiple species from different taxonomic orders were housed together at 69% of sites, only 7% of sites were reported to have domestic and wild taxa housed together. Veterinary care was available at 36% of sites. An absence of protective clothing was noted at most sites (98%), and disinfection of animal crates/equipment was rarely observed (14%). Sharing of drinking water sources between animals and humans was described at 25% of sites, with even more sharing of water for bathing.

SEASONAL EFFECTS ON THE WILDLIFE SUPPLY CHAIN

Data for each site were summarized by season, resulting in 90 sampling events in the wet season and 73 sampling events in the dry season across 126 sites, with 37 sites sampled in both seasons. There were no seasonal differences in the presence of wildlife taxa sold alive on site, but wildlife taxa were more likely to be sold dead during the wet season (59%) compared to the dry season (42%). Across all sites, drinking water was shared with animals more often in the dry season (32%) than the wet season (17%). Although insect vectors were common across all seasons (99%), vector control measures were absent at more sites during the wet season (69%) than the dry season (49%).

People and animals in the wildlife value chain, including markets, are at high-risk for viral spillover.

GENDER & LIVELIHOODS IN THE WILDLIFE SUPPLY CHAIN

We identified specific livelihood and gender distinctions. Gender is an important consideration to better understand relative zoonotic risk and inform risk communication. The gender breakdown of individuals interviewed and sampled by PREDICT teams overall was relatively even, with 52% males and 48% females working in animal production across all countries. When investigating other animal handling-related occupations, enrollment became more male-biased. Among slaughterhouse/abattoir workers (n=662), 69% were male. Of 384 individuals reporting working in the wild animal trade or wild animal markets, males made up the vast majority (79%). Among hunters interviewed by PREDICT teams (n=1,220), 72% were male. Additional questionnaire data gathered from 974 of the hunters revealed that 12% were hunting to provide live animals for sale at a market. Hunters reported capturing a wide range of animals to sell alive, including (in order of decreasing frequency) rodents, pangolins, birds, ungulates, carnivores, non-human primates, and bats. On the other hand, wildlife restaurant workers were predominantly female in Africa (90.8% out of 87 individuals), while the gender of workers in Asia was more evenly distributed (52.9% male out of the 17 individuals interviewed).

PHOTO: PREDICT GHANA

USE OF PROTECTIVE EQUIPMENT IN THE WILDLIFE SUPPLY CHAIN

Personal protective equipment (PPE) in the form of gloves, masks, gowns or aprons, and dedicated impermeable clothing and boots can substantially limit contact with infectious materials and facilitate clean up after close contact with animals. People were interviewed about their contact with animals and PPE use if they traded, hunted, trapped, or fished wild animals. Among people working in the wildlife market and supply chain, individuals were four times more likely to use PPE if they were worried about disease compared to those not worried about disease, and approximately one-third as likely to use PPE if they worked at a site with >1,000 people compared to smaller sites. Gender and education level were not significant predictors of PPE use.

Among hunters, individuals were twice as likely to have PPE if they had a secondary school education compared to no education. Hunters were also approximately twice as likely to use PPE if they were worried about disease compared to those not worried about disease, or if they had experienced an outbreak of dead wild animals in the previous year. Gender and the type of animal hunted were not significantly related to PPE use.

THE UNKNOWN THREAT: USING MODELS & MACHINE LEARNING TO EVALUATE SPILLOVER RISK

Our investigations into epidemiologic characteristics of zoonotic viruses detected prior to PREDICT surveillance revealed that wild animals were the source of zoonotic transmission for 91% of zoonotic viruses compared to 34% of viruses transmitted from domestic animals, with 25% of viruses having transmission described from both wild and domestic animals. Wild rodents were implicated as a source of spillover for 58% of zoonotic viruses, particularly for zoonotic arenaviruses and zoonotic bunyaviruses. Primates were implicated as a source of zoonotic retroviruses, while bats were more commonly implicated for zoonotic paramyxoviruses and most zoonotic rhabdoviruses.

The majority of zoonotic viruses infecting humans were found in multiple animal taxonomic orders, in addition to humans (Johnson et al., 2015). Additionally, viruses from wildlife that were detected at high-risk interfaces, including live animal markets, keeping wild animals as pets, and animal sanctuaries/zoos, were found in higher numbers of animal host taxa. This high host plasticity among both domestic and wild animal species is a result of host and virus adaptations and ample opportunity for cross-species transmission (Figure 3). Higher host plasticity among viruses transmitted by direct contact with wildlife kept as pets or in sanctuaries provides further evidence that confining taxonomically diverse species in close proximity selects for transmission of adaptable viruses with high host plasticity among wildlife. Diverse species of wild animals that are confined in zoos, sanctuaries, kept as pets and sold at markets are subject to circumstances that facilitate cross-species viral transmission via intimate contact, particularly for zoonotic viruses already adapted to transmission among domesticated animals. In many ways, high host plasticity is considered an important signal of spillover risk, especially for emerging zoonotic viruses.

ABOUT HOSTS

A **host** is the animal or plant in which an organism such as a virus infects. A reservoir host is an animal that acts as the source of infection or reinfection of other animals or people.

Host breadth is the number of possible host species for a specific pathogen or virus.

Host range is the range of cell types that a virus can infect.

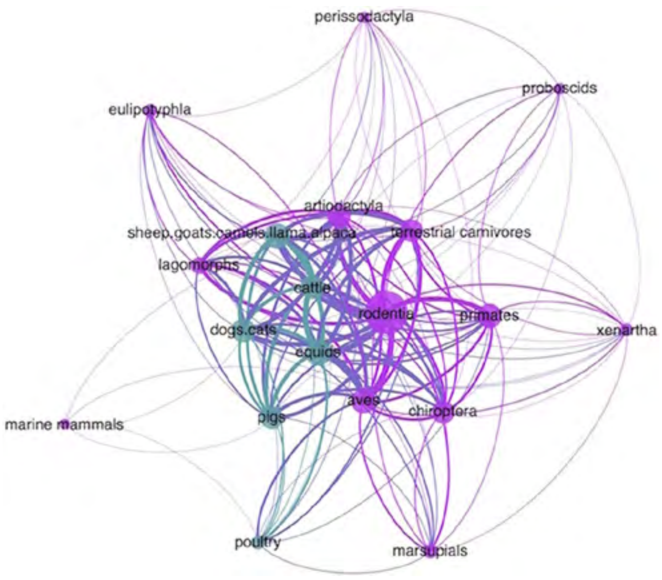


FIGURE 3. ***Host network map showing high host plasticity among zoonotic viruses with wild and domestic animal hosts connected by shared viruses (Johnson et al., 2015). Connecting line width corresponds to the number of viruses shared between hosts, and node size is proportional to number of connections each hosts has to another host, based on number of shared viruses. Wild animal hosts are in purple, and domestic animal hosts are in teal.***

WHAT IS HOST PLASTICITY?

Host plasticity refers to the number of animal species a virus is capable of infecting. High host plasticity is considered an important signal of spillover risk, especially for emerging zoonotic viruses.

By identifying patterns and propensities in the host range of well-studied viruses, we inform on potential host associations for newly discovered viruses found in similar host and ecological niches. Host-virus association patterns are becoming an established method for characterizing the host ranges of viruses (Albery et al., 2020; Gomez et al., 2013; Johnson et al., 2015; Luis et al., 2015). The structures of these host-pathogen networks are governed by underlying ecological and evolutionary processes that, for many viruses, have not yet been well-characterized (Albery et al., 2020).

From October 2009 to September 2019, PREDICT teams around the world discovered 958 novel viruses in wildlife, and the host range for these newly detected viruses was often limited to a single mammal genus, likely as a result of sampling effort for rare viruses. To better characterize these newly detected viruses, we developed machine learning models that learn from the associations formed from the hosts of known viruses. Models allow estimation of virus host ecology, including host plasticity, and their predilection towards certain taxonomic groups. A network of 269 known zoonotic and 307 non-zoonotic viruses representing 17 different virus groups and infecting 885 documented avian and mammalian hosts was used to evaluate linkages in which viruses were found in the same hosts (host sharing). Our models were used to explore potential links for 531 novel (newly detected) PREDICT viruses, to predict links between the newly detected viruses and known viruses that are formed due to sharing humans as hosts. Predictions for these newly-detected viruses generated a "predicted network" and provide insights about potential host features. Most critically, these models help identify viruses with signals of zoonotic potential (e.g., those that could evolve to pose a threat to human health) based on our current knowledge of host relationships.

PREDICT coronaviruses showed significantly higher connectivity with more species than all other viral families tested in the predicted network, indicating an ability of coronaviruses to infect a greater number of species than viruses from other groups. Given this finding, and the recent emergence of several high consequence coronaviruses, such as SARS-CoV-1, MERS, and SARS-CoV-2, high priority should be given to understanding ecological and epidemiological features of bat-borne coronaviruses, including specific genetic features that could inform on the potential for human infectivity. Novel viruses from both Paramyxoviridae and Coronaviridae families were predicted to be more likely to have human links than other viruses, emphasizing the need to further characterize the zoonotic potential of these viruses in laboratory studies.

WHO IS AT RISK? THE HUMAN DIMENSION OF VIRAL SPILLOVER

PREDICT teams in 23 countries interviewed and tested samples from 16,499 people from participating high-risk communities. Our human surveillance strategy targeted people with high-risk occupations in communities where animals were also sampled (60%), as well as acutely ill patients year-round at nearby healthcare facilities (40%). Participants completed customized data collection modules specific to their high-risk interface exposure based on their occupations or livelihoods. Modules were specific for hunters and people working in crop production, animal production, markets and animal value chains, wildlife restaurants, extractive industries, sanctuaries and zoos, and health professions, as well as people living in temporary settlements. After the module designed to document current symptoms, the most common modules completed were crop production and animal production (Figure 4).

Clinic enrollment focused on patients with acute febrile illness of unknown origin, though symptomatic individuals in the community were recruited when possible. Over 7,800 people completed the illness module, providing details on their current symptoms. About 87% of people with acute febrile symptoms were enrolled through a healthcare facility, while the remainder were recruited from the community. We tracked five groups of associated symptoms reported by participants: 1) fever with cough, 2) fever with diarrhea and/or vomiting, 3) fever with headache and/or joint pain, 4) fever with convulsions and/or altered consciousness, and 5) fever with bleeding and vomiting and/or diarrhea. Some participants met more than one symptom group criteria. The most commonly reported symptom group was fever with headache and/or joint pain, followed by fever with cough and then fever with diarrhea and/or vomiting. Fever with convulsions and/or altered consciousness was more than twice as common in Africa (10.9%) compared to Asia (4.8%). As expected, the least frequently reported set of symptoms was fever with bleeding and vomiting and/or diarrhea (<1%).

Using PCR-based detection methods targeting the four virus groups, we detected viruses in 6.0% of patients in healthcare facilities and 1.2% of people in the communities, highlighting the benefit of clinic surveillance in detecting pathogens. Coronaviruses were detected in 0.57% of clinic patients and 0.38% of community participants. Flaviviruses were detected in 1.07% of clinic patients and 0.04% of community participants. We were more than five times as likely to detect influenza viruses in patients in clinics (4.0%) than among individuals in community-based surveillance (0.74%). Paramyxoviruses were also more frequently detected in clinic patients (0.87%) than community participants (0.09%). Despite these findings, it is important to continue to target recruiting of syndromic community members to fill gaps in surveillance knowledge, particularly when access to healthcare is limited or when viruses produce mild symptoms in some individuals who may choose not to seek treatment. Our specific virus findings in people are summarized in *Chapter 2*.

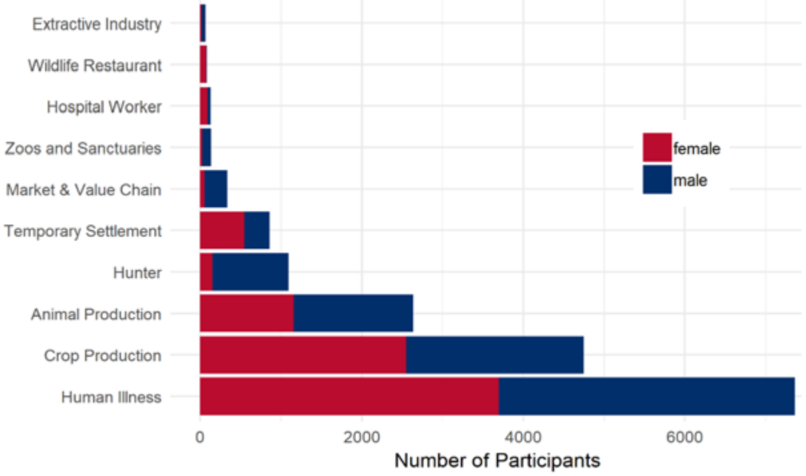


FIGURE 4. ***Number of humans sampled at high-risk interfaces from Oct. 2014 to Sept. 2019 in Asia and Africa. High-risk interface questionnaire modules were completed by individuals sampled and tested in the community or in a clinical setting, and are disaggregated here by gender. Each individual could complete more than one module and some individuals did not complete any modules.***

EXPOSED

RISKS OF VIRAL SPILLOVER

ANIMAL-HUMAN INTERACTIONS & VIRAL SPILLOVER – IS SOUTHERN CHINA A POTENTIAL HOTSPOT FOR CORONAVIRUS EMERGENCE?

Because several SARS-related coronaviruses have been discovered in bat populations in Southern China, we explored animal-human interactions that could lead to the spillover and emergence of bat coronaviruses in people. Behavioral risks such as contact and interaction with bats, are thought to be critical for the emergence of these viruses. The PREDICT China team worked with communities in the Yunnan, Guangxi, and Guangdong districts and collected samples from 1,596 individuals for serological testing. In addition, these individuals were interviewed about their interactions with animals, especially bats.

Our study found serological evidence for bat-borne coronavirus transmission to people, as nine individuals (0.6%) tested positive using our serological test, a newly developed enzyme-linked immunosorbent assay (ELISA) targeting four known bat-borne coronaviruses. The low prevalence of exposure suggests that spillover of these viruses is a rare event, and direct contact with bats was not identified as a risk factor. However, 17% of individuals self-reported severe acute respiratory infection (SARI) and/or influenza-like illness (ILI) symptoms over the past year, symptoms associated with interactions with poultry and wildlife contact (bats, rodents/shrews, and carnivores), indicating that individuals in these communities may be exposed to other zoonotic pathogens that are causing clinical illness.

PHOTO: PREDICT CHINA

Our data provide further evidence of exposure and behavioral risk to zoonotic viruses in a particularly vulnerable area of Southern China. Several coronaviruses such as SARS-CoV and SARS-CoV-2 are thought to have emerged from this area, spilling over into people and causing devastating disease. Through continued investments that integrate biological and behavioral sciences in a One Health surveillance approach that targets at-risk communities such as these, we can establish an early-warning system for non-outbreak conditions, a system that could identify emerging disease threats prior to large-scale outbreaks (Li et al., 2020).

DANGEROUS JOBS: OCCUPATIONAL RISKS FOR DISEASE EMERGENCE IN CHINA

In Guangdong Province, China, our PREDICT team, with additional funding from Google/Skoll, explored the occupational risks, behaviors, and perceptions associated with transmission of deadly pathogens at the wildlife-human interface. Our team interviewed and collected samples from 1,312 individuals with high levels of exposure to wildlife. We performed serological testing for hantaviruses, SARS-related coronaviruses, and severe fever with thrombocytopenia syndrome (SFTS) along with follow up serology on a subset of the cohort, as well as close contacts of individuals when available.

We found detectable levels of antibodies for several pathogens. Overall, individuals we interviewed reported contact with multiple species of animals. When evaluating occupational risks for infection, working as a butcher was particularly high-risk, . Interestingly, we found no evidence of increased risk of infection for professional hunters or market or restaurant workers. There was increased risk of seropositivity associated with contact with certain animals, such as civets and field rats. Very few people in the study reported using any personal protective equipment, such as gloves, when working with wild animals. In addition, there was a lack of awareness of the risks of infection for many of those interviewed; almost a third reported that they did not believe they could become infected through wildlife contact. While we did follow a subset of seropositive individuals over time, we found no evidence of infection of their close contacts, although the number of contacts we enrolled was admittedly small. Therefore, we cannot draw conclusions about transmissibility, and further investigations are needed to explore transmission risks for these pathogens.

This study illustrated that certain individuals are at higher occupational risk for viral spillover from wildlife. Butchers in particular are highly exposed to wild animals and may be influential in viral transmission, amplification, and spread. By continuing to monitor these high-risk and vulnerable groups and by integrating interventions such as use of personal protective equipment and other prevention strategies, we could effectively reduce risks for disease emergence (Monagin et al., 2018).

THE BOTTOM LINE: EXPOSURE & PERCEPTIONS OF RISK AMONG BUSHMEAT VENDORS DURING AN EBOLA OUTBREAK IN THE DEMOCRATIC REPUBLIC OF THE CONGO

In May of 2018, another Ebola virus disease (EVD) outbreak was reported in Equateur Province in the Democratic Republic of the Congo (DRC), the second in the province following the 2014 outbreak. While the origins of the outbreak remain unknown, there are assumptions that the preparation, selling, and consumption of bushmeat play a role in spillover. Our PREDICT DRC team conducted a study in Mbandaka, the capital city of Equateur, collecting samples from bushmeat and from at-risk individuals in the bushmeat value chain to explore exposure to the virus. We also interviewed bushmeat vendors about perceptions, knowledge, attitudes, and practices that might be associated with spillover risk.

We found no evidence of exposure to Ebola virus in the bushmeat samples tested. We did however detect Ebola virus antibodies in one bushmeat vendor, though this individual had no knowledge of developing the disease. From our interviews, 62% of the vendors admitted to believing EVD was real and impacting their sales (reduced sales or lower prices) during the 2018 outbreak. Regardless, 92% continued selling bushmeat during the 2018 outbreak and did not believe they were at direct risk of contracting EVD as a result of their work, which included direct daily contact with wild animal meat. Our study also reinforced that traditional beliefs continue to influence ideas about Ebola viral transmission due to a long cultural history and attribution of disease and death to witchcraft, including links with bushmeat.


PHOTO: PREDICT DR CONGO

During the 2014 and 2018 outbreaks, many of the vendors reported hearing messages on the importance of hand-washing and following this precaution before and after eating or using the bathroom, despite thinking the 2018 outbreak was not as “strong” as the one in 2014. However, when suffering cuts during butchering work, many would simply rinse their wounds with water alone; when asked about wearing protective equipment such as gloves, vendors reported that gloves often just slip off, slow them down, or get in the way. Soap was also rarely used in the cleaning of utensils or work surfaces, and there were concerns that it caused a bad, soapy taste in products that could interfere with sales.

Our findings reinforce that individuals in high-risk occupations, such as butchers and bushmeat vendors, are exposed to deadly viruses and could act as sources of spread to others in these communities. In this particular area, which has experienced multiple Ebola virus outbreaks, risk reduction and communication campaigns alone may not effectively reduce occupational hazards and risks of infection. Alongside continued messaging between outbreaks to increase knowledge and reinforce protective behaviors, we recommend further investigations into the behaviors and intervention strategies that would be effective and locally accepted and adopted. (Lucas et al., 2020).


PHOTO: GORILLA DOCTORS

BEYOND BATS: EXPOSURE TO EBOLAVIRUSES AT THE WILDLIFE-HUMAN INTERFACE IN UGANDA

The Bwindi Impenetrable Forest region in Southwestern Uganda is a mammalian biodiversity hotspot in Africa, where human livelihoods are intimately connected with wildlife. The UNESCO World Heritage Site forest is also surrounded by one of the most dense human populations in Africa, making it a particularly important interface for understanding the transmission of ebolaviruses among reservoirs, secondary spillover hosts, and humans.

Through a combination of acute febrile illness surveillance in medical facilities surrounding the Bwindi Impenetrable Forest and in-depth behavioral survey work, we were able to identify the first human exposures to ebolaviruses (Ebola virus, Sudan virus, and Bundibugyo virus) in the region, particularly those with a history of wildlife contact. The identification of antibodies to ebolaviruses in humans in Bwindi likely represents detection of previous infections with known ebolaviruses or infection with serologically cross-reactive low or non-pathogenic undiscovered filoviruses that also share wildlife hosts.

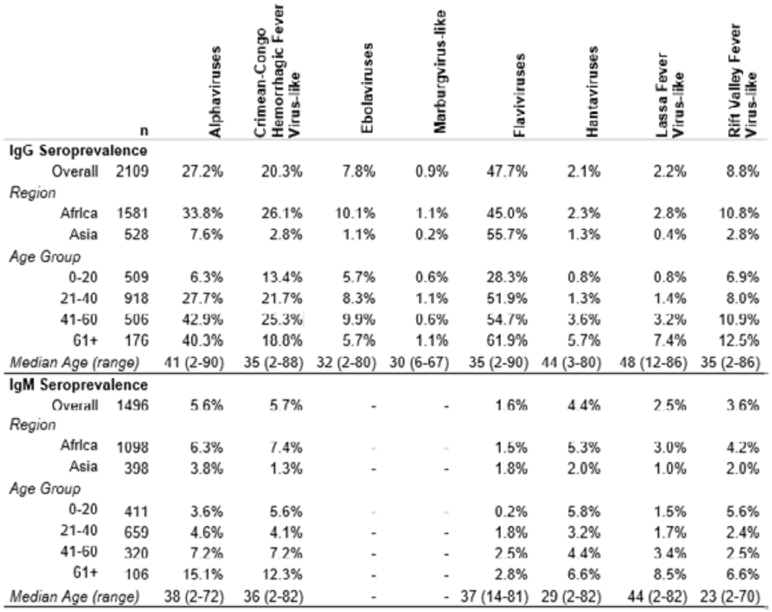
People reporting a history of hunting primates were significantly more likely to have evidence for past exposure to Sudan virus. Hunting primates has been previously linked to outbreaks of Ebola, through contact with dead common chimpanzees or western lowland gorillas. Hunting of primates may be on the increase in the region because of population growth both within Uganda and through an influx of refugees from the DRC, where primate bushmeat consumption is more common.

People with a history of touching duikers were also significantly more likely to be seropositive for Ebola virus. Duikers are frequently eaten by humans in many parts of equatorial Africa and have been previously implicated as incidental hosts for Ebola virus. Duikers are believed to become infected with Ebola virus through scavenging non-human primate carcasses, an activity that is plausible in Bwindi, given the prevalence and diversity of non-human primate species. Duikers are the most common animal caught in snares in Bwindi Impenetrable National Park, and people directly contact this species through hunting, food preparation, and consumption. Our findings indicate that spillover of ebolaviruses to humans and circulation of ebolaviruses in general could be more common than previously reported. The results of this study inform ongoing surveillance efforts needed to improve our understanding of the role of wildlife in spillover of ebolaviruses, not only adding to investigations of bats as likely reservoir hosts, but also indicating that secondary spillover host species, such as primates and duikers, may be important sources of human infection (Evans et al., 2018).

EXPLORING ADVANCEMENTS IN SEROSURVEILLANCE FOR PRIORITY ZOONOTIC VIRUSES

Scientific improvements in serology techniques and the ability to assess previous exposure to pathogens that would otherwise not be detected by PCR could greatly advance concurrent animal and human surveillance investigations and assist with characterization of risk at high-risk interfaces identified here. To promote advancements in serology, we collaborated with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) and Johns Hopkins University bon the application of recently developed serologic assays. We are also developing a novel serological platform at Columbia University targeting zoonotic and potentially zoonotic viruses detected by PREDICT teams. Known viruses within the virus groups prioritized for surveillance by PREDICT include very rare high-consequence pathogens for which serologic assays are not widely available and generally not approved for clinical diagnosis. To evaluate new serology platforms, we tested blood samples collected in clinics from patients with acute fever and additional symptoms of interest, as well as samples collected from community participants.

Given the potential for cross-reactivity in serological assays, pilot study findings available to date from USAMRIID are summarized for broad groups of viruses - specifically, alphaviruses, flaviviruses, hantaviruses, filoviruses (that include ebolaviruses and viruses similar to Marburg virus), Crimean-Congo Hemorrhagic Fever and related viruses, Lassa Fever Virus and related viruses, and Rift Valley Fever Virus and related viruses. Blood samples from over 2,100 human surveillance participants were tested for immunoglobulin G (IgG) antibodies, which can be acquired from a virus exposure anytime in a person's life, and a subset of those samples were tested for immunoglobulin M (IgM) antibodies, which indicate more recent exposure. We expect some cross-reactivity among viruses within a given viral family or genus, including novel viruses that have yet to be discovered that may or may not cause illness. Additionally, assays may interact with antibodies generated from immunization; thus seroprevalence values could include unexposed individuals that received vaccination to any closely related virus. Table 2 shows seropositivity by virus group, antibody type, region, and age group.



	n	Alphaviruses	Crimean-Congo Hemorrhagic Fever Virus-like	Ebolaviruses	Marburgvirus-like	Flaviviruses	Hantaviruses	Lassa Fever Virus-like	Rift Valley Fever Virus-like
IgG Seroprevalence									
Overall	2109	27.2%	20.3%	7.8%	0.9%	47.7%	2.1%	2.2%	8.8%
Region									
Africa	1581	33.8%	26.1%	10.1%	1.1%	45.0%	2.3%	2.8%	10.8%
Asia	528	7.6%	2.8%	1.1%	0.2%	55.7%	1.3%	0.4%	2.8%
Age Group									
0-20	509	6.3%	13.4%	5.7%	0.6%	28.3%	0.8%	0.8%	6.9%
21-40	918	27.7%	21.7%	8.3%	1.1%	51.9%	1.3%	1.4%	8.0%
41-60	506	42.9%	25.3%	9.9%	0.6%	54.7%	3.6%	3.2%	10.9%
61+	176	40.3%	18.8%	5.7%	1.1%	61.9%	5.7%	7.4%	12.5%
Median Age (range)		41 (2-90)	35 (2-88)	32 (2-80)	30 (6-67)	35 (2-90)	44 (3-80)	48 (12-86)	35 (2-86)
IgM Seroprevalence									
Overall	1496	5.6%	5.7%	-	-	1.6%	4.4%	2.5%	3.6%
Region									
Africa	1098	6.3%	7.4%	-	-	1.5%	5.3%	3.0%	4.2%
Asia	398	3.8%	1.3%	-	-	1.8%	2.0%	1.0%	2.0%
Age Group									
0-20	411	3.6%	5.6%	-	-	0.2%	5.8%	1.5%	5.6%
21-40	659	4.6%	4.1%	-	-	1.8%	3.2%	1.7%	2.4%
41-60	320	7.2%	7.2%	-	-	2.5%	4.4%	3.4%	2.5%
61+	106	15.1%	12.3%	-	-	2.8%	6.6%	8.5%	6.6%
Median Age (range)		38 (2-72)	36 (2-82)	-	-	37 (14-81)	29 (2-82)	44 (2-82)	23 (2-70)

TABLE 2. ***Summarized IgG antibody seroprevalence for eight virus groups and IgM antibody seroprevalence for six virus groups by region and age. Median age and age range are shown for positive individuals in each virus group. IgM results include only febrile participants enrolled through clinics, while IgG results include both clinic patients and community-enrolled participants. Individuals were not assessed for IgM antibodies to filoviruses.***

ON TO THE NEXT FRONTIER

PREDICT was initiated in 2009 to strengthen global capacity for detection and discovery of viruses with pandemic potential that can move between animals and people, including filoviruses, such as ebolaviruses; influenza viruses; paramyxoviruses, such as Nipah virus; and coronaviruses. Future efforts to further understand the diverse range of coronaviruses, the family to which SARS-CoV-2 belongs, are obviously urgently needed. The COVID-19 pandemic has brought the urgency of this work into our everyday lives. PREDICT activities supported emerging pandemic threat preparedness and the global health security agenda, primarily in Africa and Asia. A decade later, more than 30 countries around the world are more knowledgeable about potential pandemic threats in their countries, which animal species represent likely hosts and should be particularly avoided by people, and which animal-human interfaces present the highest risks. Additionally, these countries now have the information they need to implement or advance programs to mitigate spillover risk, and have stronger systems to detect, identify, prevent, and respond to viral threats, both known and novel. As rapid transitions in land development and anthropogenic change increase the likelihood of interactions between humans and wildlife, it is essential to continue advancing research at these high-risk interfaces to help avoid the next pandemic.

Our Consortium's findings shine a bright light on how the next Disease X will spillover. Equipped with this knowledge, we can better inform clinicians to be on the lookout for dangerous diseases and target surveillance, improving cost-effective efforts focusing on high-risk interfaces and vulnerable human and animal populations. Using new technologies and tools, such as more rapid and sensitive molecular tests, safer and more humane approaches to sampling, ongoing investigations into the human behaviors driving emergence (see ***Special Feature: What is the Wildlife-Human Interface?***), and further advances in modeling and analytics (see ***Chapter 5***), we aim to continue our quest to better characterize viral threats before they spillover.

PHOTO: PREDICT DR CONGO

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SPECIAL FEATURE

FOREST TO TABLE

THE WILDLIFE VALUE CHAIN

In a dirt-paved alley, a woman greets a visitor pulling up on a motorcycle. He unloads a few baskets from the back of the bike and brings them into a sunny room. The woman pays him, asks when the next delivery will be, then gets to work. Customers are arriving, taking seats in red, blue, and yellow plastic chairs at tables draped in vinyl cloth advertising cold local beer. In the back room, the woman opens the basket and dumps it into a bucket of water, she covers it with a lid, then a few minutes later begins preparing meals. Today's menu: rat soup, curried rat, and grilled rat – all freshly captured in rice fields and brought to town this morning.

PHOTO: PREDICT INDONESIA

What is the wildlife value chain?

Wildlife supply chains, or value chains, are complex and diverse networks that involve the removal of wild animals from their natural habitats. These animals then enter a trade network spanning a mosaic of human-dominated landscapes, often from forests and sparsely populated rural areas to dense urban markets, sometimes across borders. Live animal markets, where live wild animals and domesticated animals are sold, are embedded within this trade network. For example, the virus causing the current COVID-19 pandemic, SARS-CoV-2, was quickly traced to a live animal market in Wuhan, China that may have amplified exposure. Although there are national and international laws, regulations, and treaties governing many aspects of the wildlife trade, most traders work through a mix of legal, illegal, and unregulated means to deliver wildlife and wildlife products to end consumers. Traded wildlife come into contact with other animal species they would never encounter in the wild and are often held and transported at high densities. These stressful conditions increase susceptibility to infection and promote viral shedding, or the 'release' of virus from an infected animal often through respiratory droplets, blood, or feces.

> ***Traded wildlife come into contact with species they would never encounter in the wild and are often held and transported at high densities.***

> ***The risk of viral spillover increases as live rats are moved along the wildlife supply chain.***

INVESTIGATING SPILLOVER RISK ALONG THE WILDLIFE VALUE CHAIN IN SOUTHEAST ASIA & AFRICA

The PREDICT project focused behavioral and biological surveillance efforts on a number of wildlife supply chains involving bats, rodents, and non-human primates intended for human consumption in Africa and Asia to gain insights into the role of the wildlife trade on the emergence of viruses of pandemic potential. We explored several points of contact between wildlife and humans along these supply chains to assess the risk of viral transmission at their respective animal-human interfaces. Our findings reinforce the need for wide scale policy and human behavior change to reverse the ever-increasing trend of wildlife-human contact fueled by habitat encroachment and the harvest and trade of wildlife species.

FROM FIELD TO FORK: SPILLOVER RISK IN THE RAT TRADE IN VIET NAM

In Viet Nam, we investigated a wildlife-human trade interface that involves the capture of wild free-ranging field rats and their subsequent trade and consumption along a supply chain throughout the entire Mekong Delta region. This supply chain includes other countries, particularly Cambodia (USAID PREVENT). Driving the trade are consumers in Viet Nam and Cambodia, some of whom report eating rats at least once per week because of their good flavor, low cost, and perception of rats as 'healthy, nutritious, natural, or disease free' (USAID PREVENT).

We collected and tested samples from field rats for known and emerging viral threats. Coronaviruses were detected in multiple rat species at all 16 sites within Dong Thap province and all eight sites in Soc Trang province, with 34.6% (129/373) and 33.4% (110/329) prevalence, respectively. The overall prevalence of coronaviruses in field rats was 34.0% (239/702), ranging from 3.2% to 74.4% across sites within the two provinces (Huong et al., 2020).

We detected a very high prevalence of coronavirus positive samples in field rats intended for human consumption. These prevalence proportions increased along the supply chain (from the initial trapping of rodents, to the sale of rodents in rural and urban markets, to the consumption of rodents at restaurants), illustrating an amplification of viral transmission risk as animals move from capture sites through markets to restaurants in urban settings (Figure 1). The proportion of coronavirus positive samples was 20.7% (39/188) at the start of the value chain, 32.0% (116/363) in large markets, and 55.6% (84/151) in restaurants at the end of the value chain. Some of the rats tested were found to be co-infected with multiple coronaviruses from the *Betacoronavirus* genus, the group that also contains zoonotic coronaviruses such as MERS-CoV, SARS-CoV, and SARS-CoV-2

PHOTO: PREDICT VIET NAM

(Huong et al., 2020). Co-infection with multiple coronaviruses is an indication of emerging infectious disease risk, as hosting multiple viruses belonging to the *Betacoronavirus* genus has been shown to facilitate virus recombination, leading to the emergence of new viruses (Chu et al., 2008, Hu et al., 2017). In addition, our findings revealed that human contact with field rats increases the longer animals are maintained in captivity and when moved into more urban settings; thus, the risk of viral spillover to humans increases as live rats are moved along the wildlife supply chain. Further, the potential for contact between rodents in the supply chain and other wild and domesticated species in these urban environments offer opportunities for inter- and intra-species virus exchange and recombination; a perfect setting for the evolution and rapid spread of the next deadly virus (Huong et al., 2020).

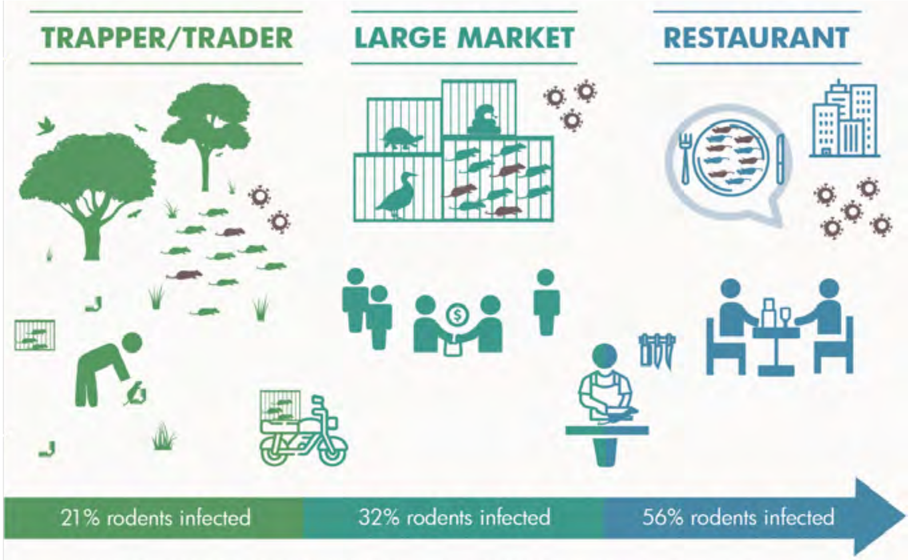

FIGURE 1. ***Increased prevalence of coronaviruses in rodents along the supply chain.***

FERTILE GROUND FOR SPILLOVER, RIPE CONDITIONS FOR SPREAD

The overall proportion of coronavirus positives found during our PREDICT project surveillance was much higher than findings from methodologically similar rodent surveys in Zhejiang province, China (2%), a previous survey in Dong Thap province, Viet Nam (4.4%), and globally (0.32%) (Anthony et al., 2017; Berto et al., 2018; Wang et al., 2015). The higher proportion of coronavirus positive samples is likely due in part to the intensity of the field rat trade with large numbers of animals moved through the supply chain at high densities. Capture and transport of wildlife, combined with overcrowding and close confinement of live animals in cages, results in increased animal contact and susceptibility to increased viral shedding. The combination of capture-related stress, dehydration, and poor nutrition to which live wildlife are subjected during transport and trade reduces animal condition and alters immune function likely contributing to both increased shedding of viruses by infected animals, and increased susceptibility to infection, as exemplified by detailed study of field rats in the value chain here.

The amplification of coronaviruses along the supply chain could vary seasonally, as field rats were significantly more likely to be positive in the wet season. For example, the ricefield rat, *Rattus argentiventer,* generally reproduce year-round in Viet Nam but are particularly abundant in the wet season (May through October) following the rice harvest when an abundance of food supports an increase in rat population size (Aplin et al., 2003). These seasonal population cycles likely affect density-dependent contact among animals, increasing coronavirus prevalence and shedding, and risk of transmission in live rats in the wildlife trade during certain times of the year.

The combination of increased coronavirus prevalence in traded wildlife, greater opportunity for wildlife-human contact as wildlife moves through the supply chain, as well as intra- and inter-species contact in trade systems is a clear driver of increased risk of zoonotic transmission of coronaviruses (and other viruses of pandemic potential) through wildlife markets, restaurants, and other interfaces along the live animal value chain. The mixing of multiple coronaviruses within a host and virus amplification along the wildlife supply chain into restaurants suggest heightened risk for end consumers and likely underpin the mechanisms of zoonotic spillover to people in these settings.

Our findings in Viet Nam have been critical to promoting dialogue among national One Health partners and the broader region regarding the public health risks associated with the wildlife trade. In the wake of the COVID-19 pandemic, government leaders in China and Viet Nam have called for bans on the trade and consumption of wildlife. Initial steps have been made in China towards significant reform of the wildlife trade to promote national and global health security.

A DIVERSITY OF RISK: TRADITIONAL MARKETS EXPOSED IN LAOS

Early PREDICT project work in Lao PDR examined the trade of wildlife within markets, where a range of species (both alive and freshly dead wild animals) are sold as food for human consumption (Greatorex et al., 2016). Surveillance confirmed that these markets, situated in provincial towns or along major roadways, are poised to facilitate spillover of viruses from wildlife to domestic animals (which are also sold through the markets) and the human visitors. Additionally, the large volumes of live wildlife (avian, mammalian, and reptilian groups) observed in these open market settings demonstrate the impact of rural to urban market trade on local and regional biodiversity. As is the case in many traditional markets where wildlife is sold, a significant number of the wildlife species observed for sale in Lao PDR are protected by national laws that prohibit their harvest from the wild. A total of 286 animals from 30 species were observed that are classified as threatened on the International Union for Conservation of Nature (IUCN) Red List, including Critically Endangered (n=1), Endangered (n=31), Vulnerable (n=206) and Near Threatened (n=48). The presence of protected wildlife species in markets highlights the weak enforcement of environmental laws and wildlife trade regulation common throughout many parts of Asia, Africa, and Latin America.

Our work in Lao PDR further examined the potential for zoonotic disease transmission from wildlife to people in these market settings by combining an analysis of the species present in the markets with the published host ranges for known zoonotic pathogens (Greatorex et al., 2016). For mammals that were live or freshly dead, 21 genera from 12 wildlife families were observed that have the potential to host 36 significant zoonotic pathogens, including those associated with diseases, such as rabies, SARS, leptospirosis, and *Mycobacterium tuberculosis* complex. The 12 wildlife families were Muridae (rat species; potential to host 26 significant zoonoses), Suidae (wild pig; 18), Pteropodidae (fruit bats; 17), Sciuridae (tree and flying squirrels; 15), Cervidae (muntjac, sambar; 15), Leporidae (hare, 15), Felidae (leopard cat; 14), Rhinolophidae (insectivorous bats; 9), Viverridae (civets; 7), Herpestidae (mongoose; 3), Hystricidae (porcupine; 3), and Lorisidae (loris; 1). Moreover, five of these mammalian families that are capable of hosting a high number (7 to 26) of significant zoonoses were in the markets in large volumes (greater than 100 individuals or kg per family): Sciuridae, Pteropodidae, Muridae, Cervidae, and Viverridae – a testament to the high-risk disease interfaces these market create.

PHOTOS: PREDICT VIET NAM & INDONESIA

What's on the menu?

WILD MEAT IN INDONESIA

The PREDICT team investigated the wildlife trade networks in Sulawesi, Indonesia (Latinne et al., 2020), with a focus on the wildlife trade in North Sulawesi province. Field investigations and ethnographic interviews with hunters, vendors, and trade intermediaries revealed an organized and dynamic trade network involving many actors. The studies revealed that the trade for wild meat has increased substantially since the early 2000s, a trend that is likely mirrored across the region, as increasingly affluent human populations drive demand for wild meat consumed as a luxury item. Analyses of wild meat prices in both Laos and Sulawesi confirmed that wildlife meat is more expensive than market prices for meat from domestic species, and its purchase is often associated with special occasions. As in Lao PDR, investigations in Indonesia included observations of and data collection on protected species of wildlife on sale in markets, as well as the sale and trade of species for which no quotas for commercial trade had been established. The wildlife groups most commonly found in the Sulawesi trade were bats, wild pigs, rats, and snakes.

> ***"Affluent human populations drive demand for wild meat consumed as a luxury item."***

Estimates of the total annual quantities of wildlife sold through Sulawesi markets (not including supermarkets or wildlife restaurants) were 319 tons of bats, 419 tons of wild pigs, and 72 tons of snakes. Specifically, the quantity of bats sold through Sulawesi markets was estimated to be equivalent to over 600,000 individuals and possibly up to one million. An in-depth study into flying fox (fruit bat) hunting revealed that current rates of harvests are clearly unsustainable. Population declines have already affected the availability of wild meat, and hunting that originally occurred in the north of the island has since expanded to the central and southern provinces to supply the market demand in the north (Lee et al., 2005; Sheherazade & Tsang, 2015; Latinne et al., 2020).

PHOTO: PREDICT INDONESIA

RAISING RODENTS OR SPILLOVER RISK? WILDLIFE FARMS IN VIET NAM

Commercial wildlife farming in Viet Nam is part of the expanded international trade of wildlife that contributes to the increasing risk of epidemics, such as SARS (Swift et al., 2007). Over the past three decades, commercial wildlife farming has developed in many countries in Southeast Asia, including Viet Nam, where the PREDICT project focused both biological and behavioral surveillance activities around the wildlife farm interface. Although there are historic references to the occurrence of wildlife farms in Viet Nam dating back to the late 1800s, we documented rapid expansion in terms of farm numbers, species diversity, and scale of operations in recent decades in response to growing domestic and international demand for wildlife (WCS, 2008). A 2014 survey across 12 provinces in southern Viet Nam identified 6,006 registered wildlife farms of which 4,099 had active operations. The surveyed farms were stocked with approximately one million wild animals including rodents, primates, civets, wild boar, Oriental rat-snakes, deer, crocodiles, and softshell turtles. Ninety-five percent of the farms held one or two species of wildlife, and 70% of the farms also raised domestic animals on the same premises (FAO, 2014). A key component of the wildlife farm industry in Viet Nam is the raising of wild species for meat for human consumption (FAO, 2014). These farms sell to urban wild meat restaurants, which serve increasingly affluent populations throughout the country, and also supply international markets with wild meat (Robertson et al., 2003).

Through our ethnographic interviews and focus group discussions, we engaged wildlife farmers and other actors involved in the wildlife farming trade network in Viet Nam, revealing a range of destinations for farm-raised wildlife including other farms, markets, wildlife restaurants, and sales to international traders (Figure 1). Wildlife breeding stock on farms were sourced from other farms, as well as from traders who captured animals from the wild (Figure 2). No quarantine procedures on farms were reported, and little to no general disease control measures were practiced. Most wildlife farmers discussed raising other species on their farms (multiple wildlife species or a mix of domestic and wild species). Wildlife farmers also reported purchasing domestic animals (and in some cases other wildlife) to feed wildlife stock (e.g. rats purchased to feed to snakes).

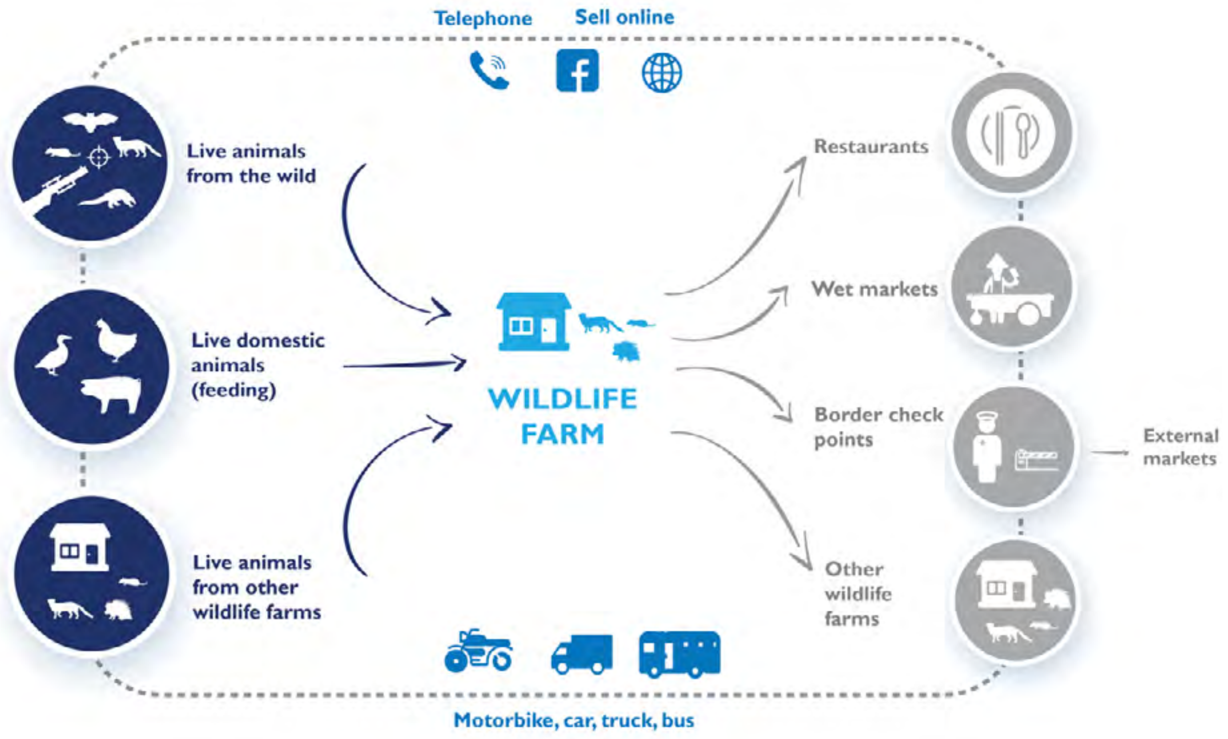

FIGURE 2. ***A model of the wildlife farm and trade network in Viet Nam.***

Our surveillance on wildlife farms in Viet Nam focused on rodents, civets, and non-human primates and resulted in the detection of multiple known and novel viruses, the majority of which were coronaviruses. A number of viruses were found in multiple species suggesting cross-species transmission is occurring on wildlife farms. Known coronaviruses of rodents were found in farmed, wild species raised for human consumption. A bat-origin coronavirus was found in the feces of Malayan porcupines, Bamboo rats, and a free-ranging rat on the wildlife farms surveyed. A novel rhabdovirus was found both in rodents and macaques, and a coronavirus known to infect poultry (infectious bronchitis virus, IBV) was found in environmental and fecal samples collected from Malayan porcupines and Bamboo rats. It is unclear if the Malayan porcupine fecal samples were infected with the avian or bat viruses, or if environmental contamination or mixing occurred with avian and bat guano. Chickens were present at the two sites where the IBV-positive rodents were detected, and bats are present and potentially roost at most farms.

With the mix of species raised, the generally standard practice of raising animals in confinement, and the limited feasibility of instituting biosecurity measures on these wildlife farms, it is not surprising that animals are exposed to a mix of viruses in their environment and that cross-species transmission can and does occur. The majority of viruses we identified on the farms were in wildlife raised for human consumption. This finding raises major concerns about food safety and the emergence of viruses of pandemic potential along commercial supply chains for wildlife meat. Wildlife farms clearly need to be considered in wildlife trade law and policy reform; these are very vulnerable interfaces that threaten health security.

> *Wildlife farms are very vulnerable interfaces that threaten health security.*

PHOTOS: PREDICT REPUBLIC OF THE CONGO

RISK OF ZOONOTIC SPILLOVER & SPREAD AT WILDLIFE MARKETS IN CENTRAL AFRICA

We conducted an in-depth characterization of wild animals for sale in markets in Cameroon and the Democratic Republic of the Congo (DRC). Using daily observation data collected from May-September 2018, we assessed trends in the diversity of animals found in the markets. A wide range of wildlife and animal products including live, freshly killed, smoked, and frozen animal carcasses and wild animal parts were observed. Wild mammalian species observed for sale included carnivores, non-human primates, pangolins, rodents, and ungulates. In addition to wild mammals, we observed reptiles (snakes, turtles, and tortoises) and, infrequently, pigs and wild birds available for sale across markets.

In assessing the condition of all mammals sold at the markets in Cameroon, a majority of the animals were sold freshly butchered. Pangolins were most commonly observed alive, while non-human primates rarely were. Rodents and carnivores were always recorded dead. The most common species observed in Cameroon included ungulates (bay duiker, blue duiker), rodents (African brush-tailed porcupine, marsh-cane rats), pangolins (long-tailed pangolin, tree pangolin), and non-human primates (*Cercopithecus* genus).

A diversity of wildlife was also recorded in markets in DRC. In contrast to observations in Cameroon, primates sold at markets in DRC were more commonly alive at the time of sale (97% of primates in one market and 17% of primates in another market). Commonly observed non-human primate species included red-tailed guenons, Allen's swamp monkeys, and several mangabey species. Marsh cane-rats were usually sold already slaughtered, with 16% observed alive at the time of sale. Other wildlife included ungulates (which could not be identified to the species level), carnivores (African civet, servaline genet), and pangolins (also could not be identified to the species level), although the latter were rarely observed.

Non-human primate, rodent, and ungulate species that are known hosts of zoonotic viruses from multiple virus groups were present in the markets (Stephens et al., 2017). Of the animals observed at both the DRC and Cameroon markets, 11 species have been previously associated with viruses transmitted through arthropod vectors including bunyaviruses (Bwamba virus), flaviviruses (Zika and yellow fever viruses), and togaviruses (Chikungunya). We also observed 14 non-human primate species that have been associated with retroviruses such as simian foamy virus (SFV) and simian immunodeficiency virus (SIV), as well as duiker species that have been associated with Ebola virus (Smiley Evans et al., 2018).

Live wildlife have the potential to transmit zoonotic viruses to people through close contact during handling and slaughter. Given frequent contact with blood, tissue, urine, feces, and respiratory droplets, as well as the potential for cuts, bites, and scratches, disease transmission from animals to people is a likely occurrence wherever wild animals are kept, slaughtered, and sold.

There are many opportunities for disease spread as animals and people move between local, regional, and international live animal markets.

DUAL THREAT REDUCTION FOR A HEALTHIER PLANET

Live animals are butchered in markets, often with limited to no biosafety or hygiene practices in place that protect people handling animals or that contain the spread of potentially deadly pathogens. These markets trade a large number of wild animals from high-risk animal groups known to carry dangerous zoonoses, thus there are many opportunities for disease spread, as animals and people move between local, regional, and international live animal markets. These factors put us at risk for repeating yet another global health crisis.

Partners in the PREDICT Consortium are among the world's leading experts supporting evidence-based policy at the state, national, and international scale. We worked with One Health platforms at all levels to highlight the role that wildlife markets and wildlife trade have and will likely continue to play in disease emergence if significant reform is not realized. The dual threat of the wildlife trade to public health and the conservation of biodiversity locally and globally requires multiple agencies and stakeholder groups (including law enforcement officers, commerce and trade authorities, public health inspectors, and veterinarians) to join forces to support communities and nations to phase out dangerous wildlife trade practices. The COVID-19 pandemic has brought much needed public and policy attention to the risks associated with trade in live wild animals, but action is still required.

PHOTO: PREDICT CHINA

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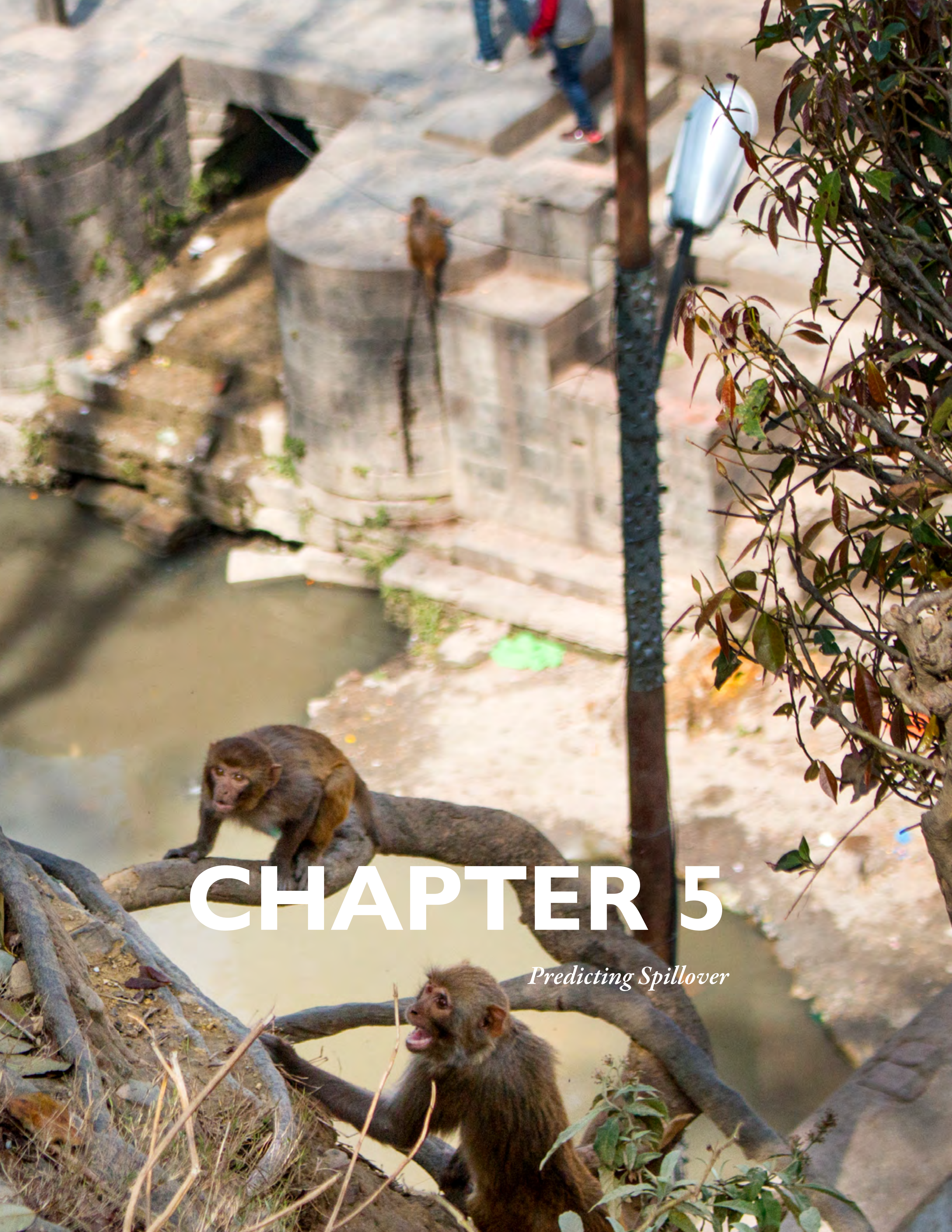
PHOTO: PRANAV PANDIT



CHAPTER 5

Predicting Spillover

KEY TAKEAWAYS

- The PREDICT project built a deep understanding of which geographies, species, animal-human interfaces, and behaviors represent a high risk for future disease emergence.
- We pioneered new modeling strategies and open-source tools to analyze the risk of disease spillover, amplification, and spread, and to push the field of viral forecasting forward.
- We are better prepared to identify and map the risk of newly emerging diseases (i.e. Disease X) and also the clear and present threat of known deadly pathogens, such as Ebola virus, Nipah virus, and SARS-related and MERS-related coronaviruses.
- Field data from around the world and mathematical models were combined to iteratively refine surveillance targets and improve the efficiency of virus discovery efforts over the life of the project.
- Our modeling and analytics experts worked collaboratively with our in-country partners to train and support their capacity for risk analyses and to make the tools, analytical products, and data outputs available to national decision makers.

What if we could forecast disease emergence and take action to preemptively stop spillover?

It's dawn in rural northern Tanzania. The sun is peaking over the forested green peaks lighting up the hills, the maize fields, cattle, goats, and sheep grazing, the dirt-paved village streets. In one home, as tea and breakfast are prepared over the gas stove and mosquitoes buzz around, a cellphone vibrates on a nearby table. A young girl grabs the phone and reads the alert, it's a warning from the local ministry of health, part of the national emergency messaging system: "ALERTTZ: Conditions today remain risky: The forecast is orange/medium to high-risk for Rift Valley Fever spillover. Take precautions: https://spilloveralert.RVF_92120#ffzzz."

A BOLD VISION: THE END OF THE PANDEMIC ERA

PHOTO: NIGEL WALKER

The PREDICT project's vision was bold and ambitious – to build capacity, gather data, and conduct surveillance in wildlife and people at critical high-risk interfaces so we could better predict and ultimately prevent pandemics. Our focus was on stopping pandemic emergence at the first event – the 'spillover' from wildlife to livestock or people.

If successful, we could disrupt the chain of disease transmission, snuffing out an outbreak before the first case amplified into a local epidemic and then spread internationally. But to stop spillover you have to know when, where, and how it will happen; you need diverse types of data and new approaches in order to forecast outbreaks.

HOW DO YOU PREDICT SPILLOVER?

Accurate prediction is dependent on quality data and information. We rely on predictive models and forecasts to make everyday decisions. Will it rain today, should we reschedule the picnic? What's the risk of a wildfire in my area, and should I be on alert? Are cases of COVID-19 rising in a community, do we need to ramp up test and trace efforts or close down non-essential businesses, restaurants, schools, and parks?

Predicting viral spillover or disease emergence is no different. Accurate predictions require quality data and robust models with underlying assumptions grounded firmly by evidence. Through PREDICT, we used our project's vast ecological, environmental, sampling, and testing data, along with the latest advances in analytics, modeling, and mapping to move the needle towards a future in which we might accurately devise disease emergence forecasts and a warning system to prevent pandemics.

Over the last 11 years, the PREDICT Consortium has vastly accelerated the field of spillover and emerging disease prediction. We developed new analytical and modeling strategies and open-source tools to analyze the risk of spillover, amplification, and spread. Our modeling and analytics experts worked collaboratively with our in-country partners to train and support their capacity for risk analyses and to make the tools, analytical products, and data outputs available responsively to requests from their national ministries. We developed and trained the Consortium on open-source statistical data packages that integrate with our project's data platform to facilitate data cleaning and collaborative analysis by in-country teams and global staff. We worked directly with national agencies, ministries, and other stakeholders to produce maps, models, and other products that help design comprehensive disease surveillance programs, test hypotheses and assumptions, and hone national One Health strategic plans. Across a broader context, we collaborated with the United Nations Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE), and the World Health Organization (WHO), including analyzing future trajectories of disease risk based on rapidly developing livestock production capacity in African countries.

The PREDICT project has led the way in multi-disciplinary One Health analyses to predict and prevent emerging infectious diseases by harnessing the diverse expertise of our team in areas of disease ecology, spatial analysis, epidemiology, evolutionary biology, virology, computer and data science, and behavioral research. The robust data we collected, including ecological and site characteristics, virus detections, virus sequence data, host species distributions, and human behavioral risk data, was harnessed to increase the efficiency of our One Health surveillance efforts and fine-tune our targets in real-time (see *Chapter 1*). The analyses and tools that we developed over the last decade include global approaches to identify and map the risks of newly emerging diseases (i.e. Disease X) and better understand the clear and present threat of known deadly pathogens, such as Ebola virus, Nipah virus, and SARS-related and MERS-related coronaviruses.

Here we highlight key findings from over a decade (2009-2020) of our work to predict where, why, when, and how zoonotic diseases will emerge; the foundation of a future spillover forecast for pandemic prevention.

PHOTO: PREDICT UGANDA

WHY ARE DISEASES EMERGING?

Just before the start of the PREDICT project in 2009, Consortium members published the first robust global analysis of patterns and trends in emerging diseases, correcting for underlying variability in surveillance effort. The resulting paper (Jones et al., 2008) showed that emerging infectious disease (EID) events are occurring more frequently, particularly those originating in animals. Beginning in 2010, PREDICT began to update the underlying data on past emerging disease events and created the open-source and interactive Emerging Infectious Disease Repository (EIDR) to house this critical information. In 2017, the PREDICT team used these data and a machine learning technique to completely overhaul the EID hotspot analysis, improve correction of underlying reporting biases, and use new and updated datasets to better understand the ecological, biogeographic, and anthropogenic drivers of the most important EIDs – those caused by zoonotic diseases (Allen et al., 2017). In Figure 1 we ranked the most important drivers of spillover from this work. Our results highlight the importance of mammal biodiversity as a source of new microbes that cause zoonoses and of human demographic factors, such as population density and growth. Importantly, they also clearly point to global environmental change (e.g. converting forest to pasture) as a key part of the process of disease emergence globally.

> *Global environmental change (e.g. converting forest to pasture) is a key part of the process of disease emergence globally.*

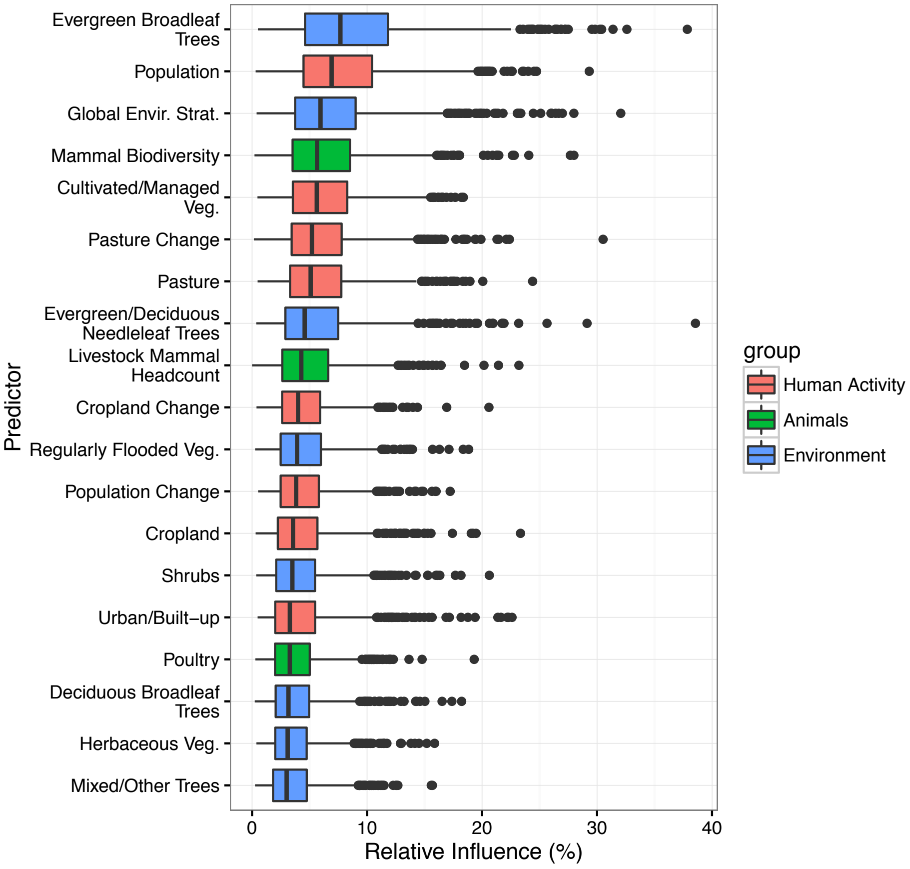


FIGURE 1. ***The relative influence of ecological and demographic predictors for zoonotic disease emergence, ranked from top to bottom. The box plots show the spread of relative influence across 1,000 replicate model runs to account for uncertainty in EID event location (see above). From Allen et al., 2017.***

Using complementary network modeling, we also ranked disease transmission interfaces and the key ecological and epidemiological processes influencing the evolution, spillover, amplification, and spread of viral threats (Johnson et al., 2015). This allowed us to identify additional, more finely tuned risk factors, including animal contact around human dwellings, hunting and consumption of wildlife, and ecotourism (Figure 2). This also allowed us to focus our global approach into direct benefits for PREDICT's surveillance and data collection.

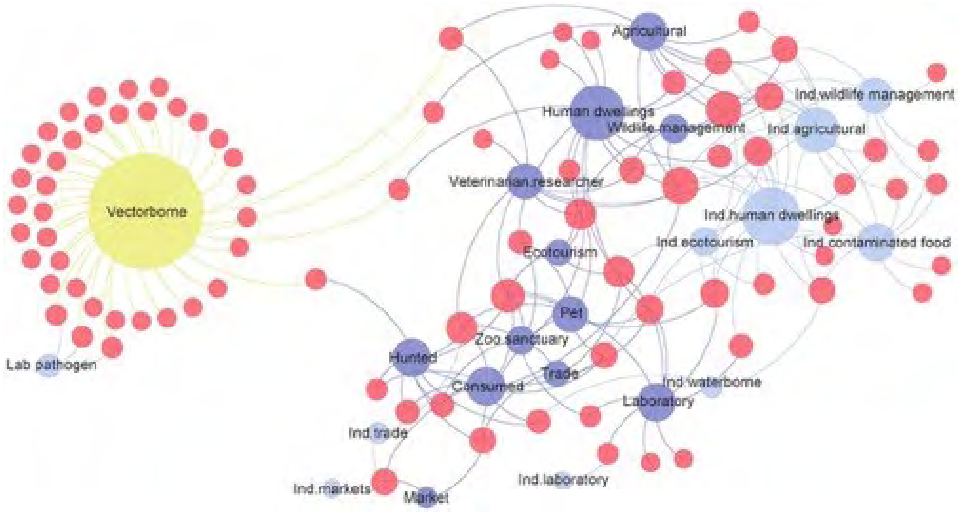


FIGURE 2. ***Network map of high-risk disease transmission interfaces shared by wildlife-to-human transmitted zoonotic viruses. Node (circle) size proportionate to the number of viruses reported at each transmission interface. Direct contact with wildlife (dark blue), indirect contact with wildlife (light blue), transmission by vector (yellow). Highly connected interfaces transmit more viruses, likely lead to higher rates of disease emergence and are therefore key targets for surveillance and EID prevention.***

To further dissect the roles of land use, human behavior, and wildlife diversity in driving disease emergence, we coordinated data collection across a land use gradient and analyzed virus diversity in a transformative collaborative effort, the DEEP FOREST project, which we conducted in Brazil, Uganda, and Malaysia. We calculated the spillover risk across the landscape (Figure 3) based on the diversity of wildlife and the viruses they harbor (the pathogen pool) and the contact behavior of people (their exposure).

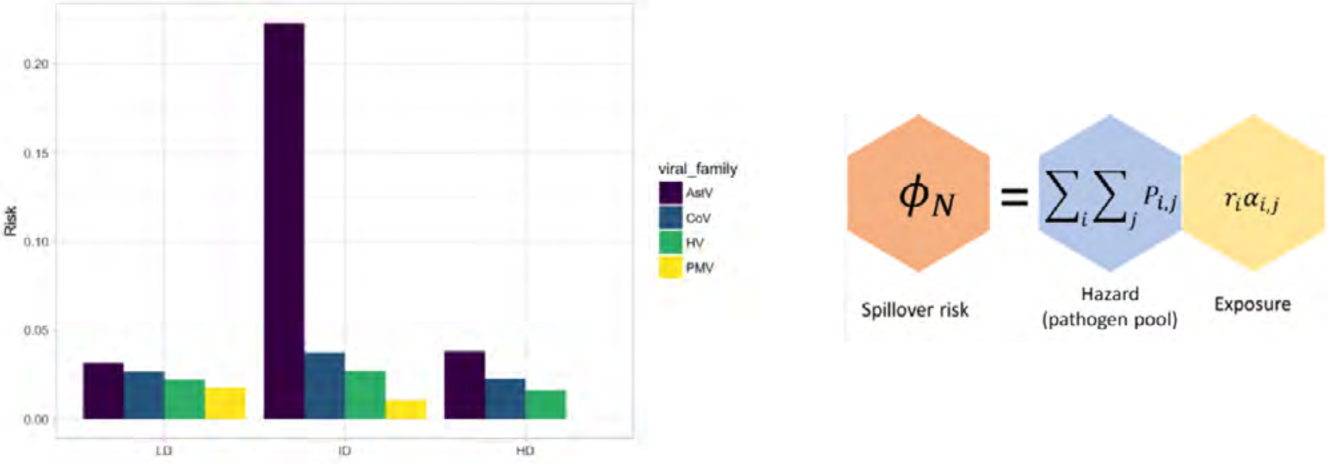


FIGURE 3. ***DEEP FOREST analysis of how spillover risk varies across a land use gradient (low disturbance, intermediate disturbance, and high disturbance) for four viral families (astroviruses (AsV), coronaviruses (CoV), herpesviruses (HV), and paramyxoviruses (PMV) in Malaysia.***

Results showed that risk of spillover was highest at the intermediate levels of disturbance (Figure 3), at sites where deforestation has already begun to transform the landscape, but there is still a relatively high diversity of wildlife. Here, increasing numbers of people make contact with wildlife with a higher number of pathogens and drive a higher risk of pathogen spillover. These analyses take into account data from standardized behavioral questionnaires to quantify wildlife-human interactions and wildlife consumption (Figure 4). Overall, less wildlife consumption was reported in Uganda than in Brazil or Malaysia. Additionally, less wildlife consumption was reported in the urban sites compared to the semi-disturbed and pristine sites in the two countries reporting high wildlife consumption (Brazil and Malaysia). This finding alone highlights how wildlife consumption varies in different cultural contexts and illustrates why all potentially high-risk behaviors should be explored across geographic and cultural zones.

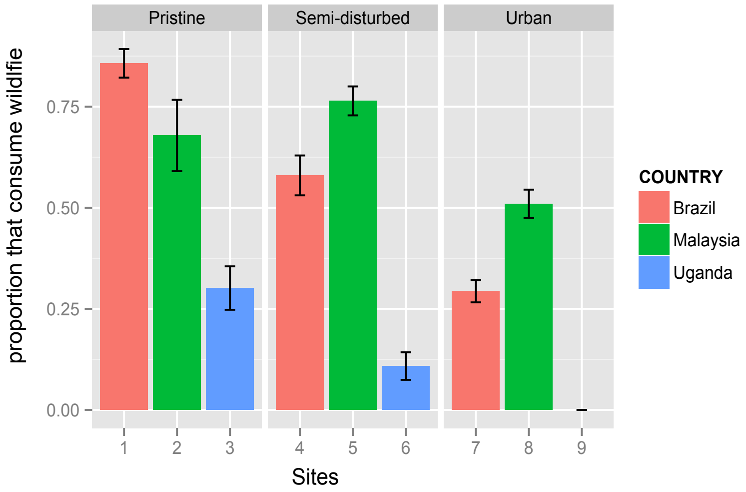


FIGURE 4. ***Proportion of respondents reporting wildlife consumption across three countries (and continents) and three gradient levels. Error bars at 95% confidence intervals around the estimated proportion of respondents reporting wildlife consumption. Data show that eating wildlife is more common in pristine habitats, and overall more frequent in Malaysia as compared to Brazil and Uganda.***

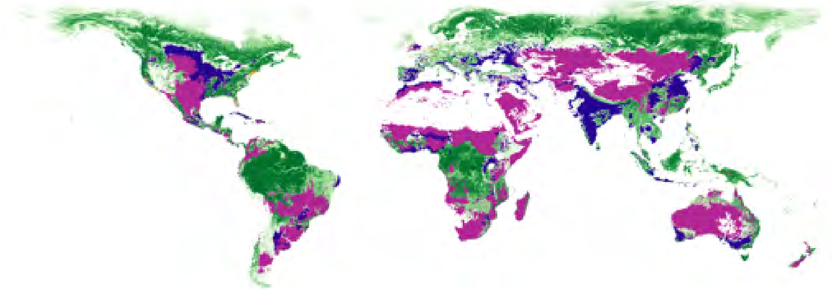


FIGURE 5. ***Global changes in land use (urban, pasture, and cropland) from 1970 and 2005. Regions with the greatest change have a higher risk of disease emergence.***

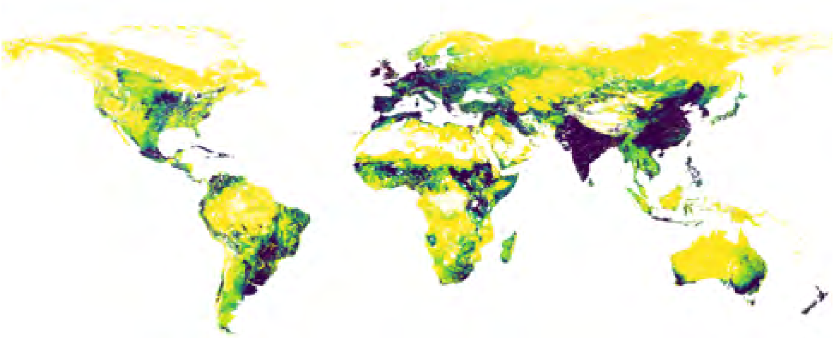


FIGURE 6. ***Map of global mammalian livestock density. Livestock often act as "bridge hosts" allowing spillover of pathogens from wildlife to people. Here, combined population density of buffaloes, cattle, goats, pigs, and sheep are derived from an FAO model that combine animal census data with predictors of livestock density, including several vegetation, climate, topography, and demography variables.***

USING OUR DATA TO INFLUENCE PANDEMIC PREVENTION POLICIES

Our modeling and analytics showed that four key factors underpin disease emergence: 1) land use change (urbanization, deforestation, changes to cropland and pasture), 2) wildlife diversity, 3) livestock density, and 4) human population density. To make our insights more useful to policy makers, national ministries, agencies, and other stakeholders, we worked closely with USAID Emerging Pandemic Threats program partners to update, aggregate, map, and visualize global datasets for landuse change and livestock production (Figures 5-7). Country-level visualizations for each of these maps were shared with PREDICT country teams during a risk mapping exercise at our annual meeting in 2018 (see *Chapter 7*). We also closely collaborated with FAO on the Africa Sustainable Livestock 2050 (ASL2050) project to specifically model the risk of disease emergence in the face of expanding future livestock production in Africa.

Four key factors underpin disease emergence: land use change, wildlife diversity, and livestock and human population density.

Land use change in many low and middle income countries (LMICs) is driven largely by the sale of timber and the use of converted land for profitable agriculture, including livestock production and valuable crops like oil palm. The Infectious Disease Emergence and the Economics of Altered Landscapes (IDEEAL) project examined the value of palm oil production versus the cost of diseases that emerge when land use change gradually replaces a tropical forest landscape over decades of development. IDEEAL initially focused on Sabah, Malaysia as a pilot site, then expanded to Peninsular Malaysia and Thailand, with additional engagement in Sarawak and Indonesia during the project extension. An Indonesian counterpart was developed in close collaboration with the Indonesia One Health University Network (INDOHUN) as part of the EPT's One Health Workforce program. IDEEAL involved detailed calculation of the costs of zoonotic malaria, including days off work, rates and cost of hospitalization, cost of malaria control programs, and correlation between land use change and malaria caseloads.

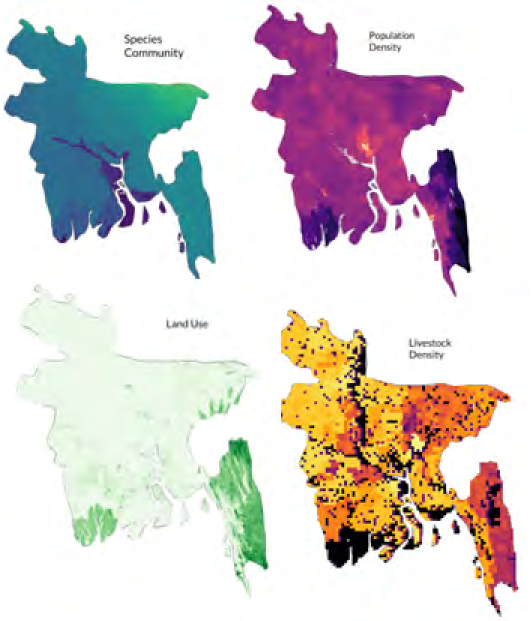


FIGURE 7. ***Example from Bangladesh shows one downscaled, country-level map of key contributing factors to zoonotic disease risk. Maps like these were shared with each PREDICT country team and other stakeholders to facilitate discussions around national zoonotic disease surveillance priorities.***

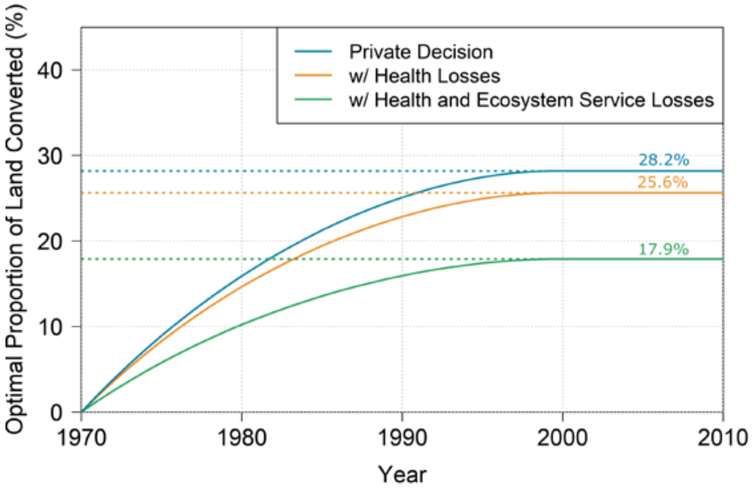


FIGURE 8. ***Comparison between conversion of historical forest over the last four decades (horizontal axis) vs. optimal economic production of land converted for palm oil (vertical axis). The blue line is the optimal path for deforestation if no ecological costs are accounted (ecosystem services). The orange line is when the economic losses due to malaria are included. The green line is where both health and ecosystem services are incorporated.***

The results from IDEEAL (Figure 8) show that ignoring the proven relationshiop between malaria cases and deforestation leads to over-conversion, the loss of important ecosystem services, and significant public health costs from disease prevention, treatment, and loss of life. In Sabah, Malaysia, the economic loss due to increased malaria incidence through excessive land conversion over the past 30 years is ~$748 million, or an average of ~$25 million annually. This corresponds to 0.13% of Sabah's GDP or $18 per hectare per year just when malaria is considered, not accounting for the many other EIDs that are known to be linked to land use change. Through the PREDICT project, we also developed a global, country-level model that estimated total economic loss of future EIDs due to excessive global land conversion over the next 30 years: $18 and $52 billion. IDEEAL engaged over 1,000 individuals over the course of the project and brought more than 40 stakeholder groups from communities, governments, universities, and private companies to the same table to discuss the potential health impacts of land use change.

We developed toolkits for science communication and engagement and hosted trainings and work shops in multiple countries in Asia, as well as the USA, on topics including: modelling (intro to R, a statistical software program, and Geographic Information Systems, GIS), zoonotic disease and economics, zoonotic disease surveillance, health and land use planning and management (IDEEAL toolkit), and communication skills (funding science and knowledge translation).

> ***In Sabah, Malaysia, the economic loss due to increased malaria incidence through excessive land conversion over the past 30 years is ~$748 million.***

WHERE WILL DISEASE X SPILLOVER? WHERE WILL IT SPREAD?

The risk of a new zoonotic disease emergence event (Disease X) is not uniform around the world. Our updated EID Hotspots 2.0 analysis published in *Nature Communications* in 2017 (Allen et al., 2017) showed that zoonotic disease spillover risk is greatest in tropical regions with high levels of biodiversity, expanding human populations, and rapid ecological change (Figure 9). Outputs of these models and code are publicly available, allowing researchers around the world to access this important information. Further, these maps were downscaled to a finer resolution for each PREDICT country and shared with our country teams as a surveillance planning tool (Figure 10).

Zoonotic disease spillover risk is greatest in tropical regions with high levels of biodiversity, expanding human populations, and rapid ecological change

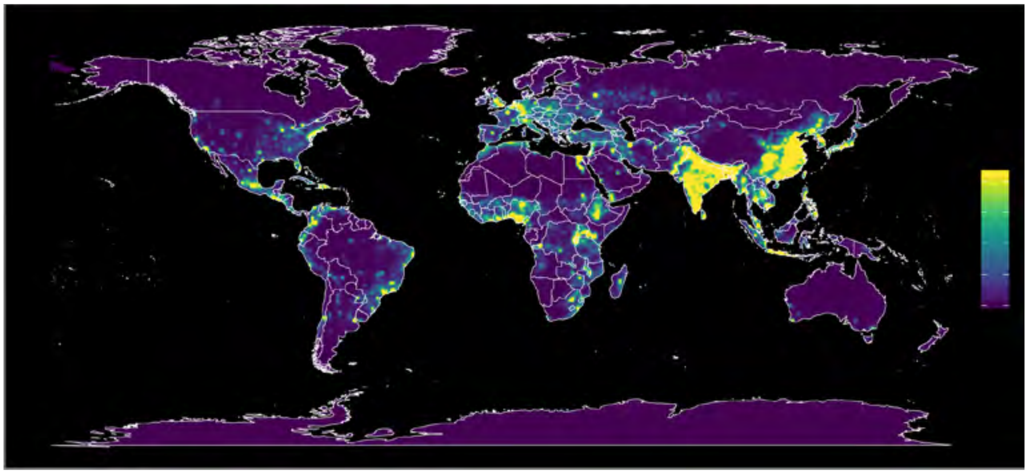


FIGURE 9. ***Hotspot map showing the relative risk of a new emerging zoonotic disease. Yellow = highest, purple = lowest risk. This map is corrected for global variations in reporting effort to give a true measure geographic spillover risk. Model constructed using variables from Figure 1.***

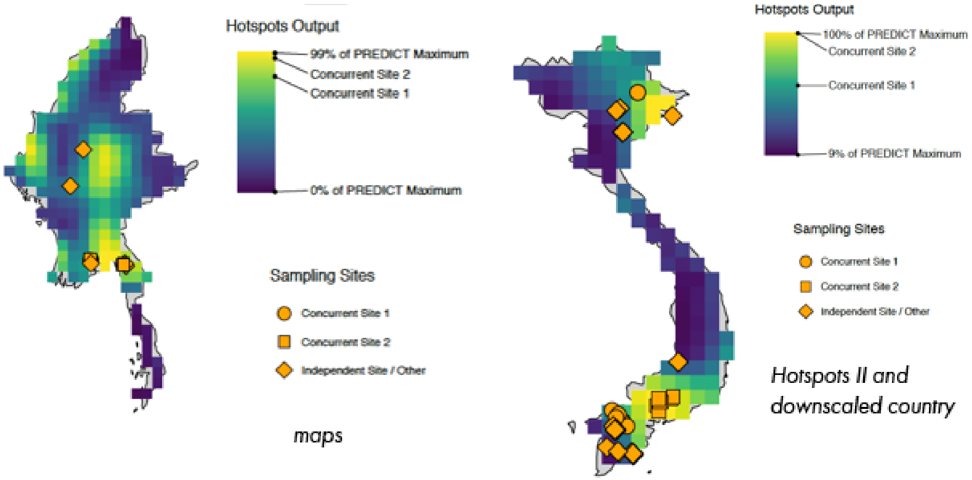


FIGURE 10. ***Spatial zoonotic risk (hotspot) maps were presented to each of the 28 PREDICT country teams at the all-country meeting in 2018 to give higher resolution maps for in-country use and surveillance planning, examples shown for Myanmar and Vietnam with PREDICT sampling sites overlaid on the spatial risk projections.***

HOW CAN WE FILL DATA GAPS FOR MISSING ZOONOSES?

Maximizing the efficient discovery of new viruses in wildlife is challenging because historical data on sampling is biased geographically (places with easier access predominate) and taxonomically (larger easier-to-hunt species predominate). We produced a global model of virus discovery and host taxonomy data, coupled with the latest information from the International Union for the Conservation of Nature (IUCN) on species distribution for every mammal on the planet (Olival et al., 2017) (Figure 11). By setting global surveillance intensity across all species at the same level as the most studied wild mammal (the red fox), we were able to generate estimates and maps of relative degrees of missing, or yet-to-be-discovered, viruses and zoonoses (Figure 12). These "missing zoonoses" maps were downscaled and expanded to forecast viral richness for all mammals globally and distributed to PREDICT country teams as a planning tool and risk mapping exercise. In several cases, these maps were used to shape national surveillance plans for pathogen identification and risk reduction, for example to prioritize sampling areas for the Thailand National Virome Project in 2019.

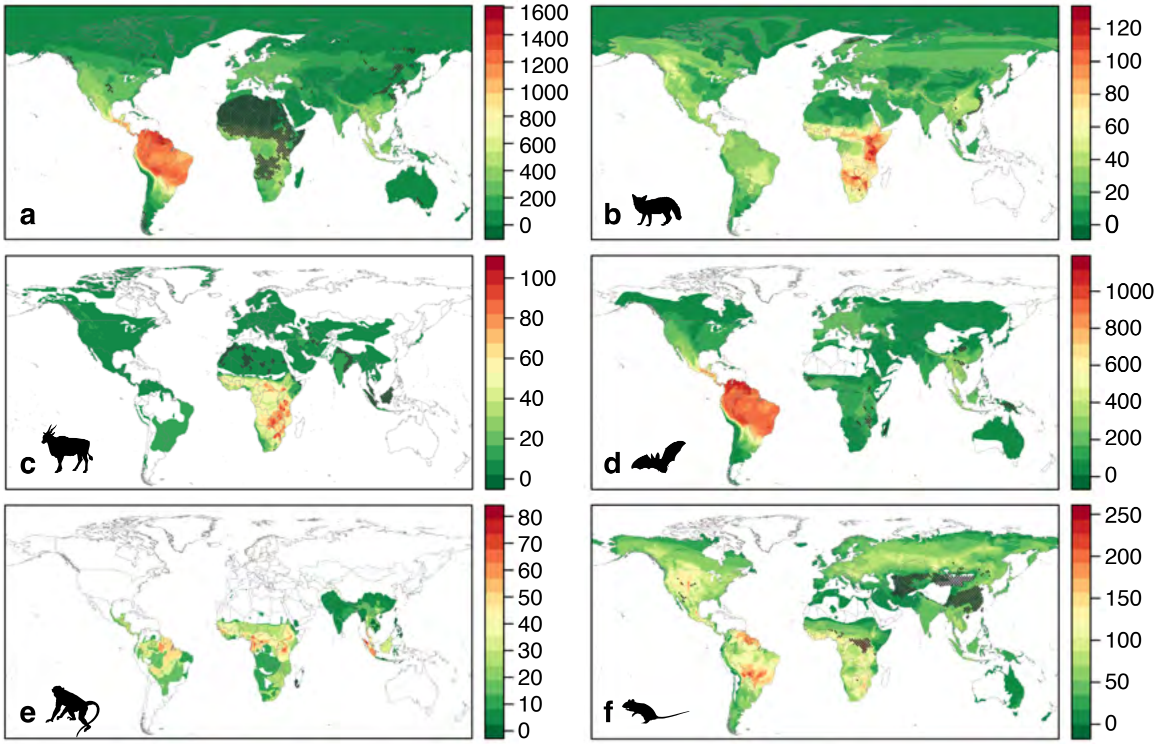


FIGURE 11. ***Global distribution of the predicted number of 'missing zoonoses' by mammal order. Warmer colors highlight areas predicted to have the highest diversity of novel, as-of-yet discovered viruses with zoonotic potential.***

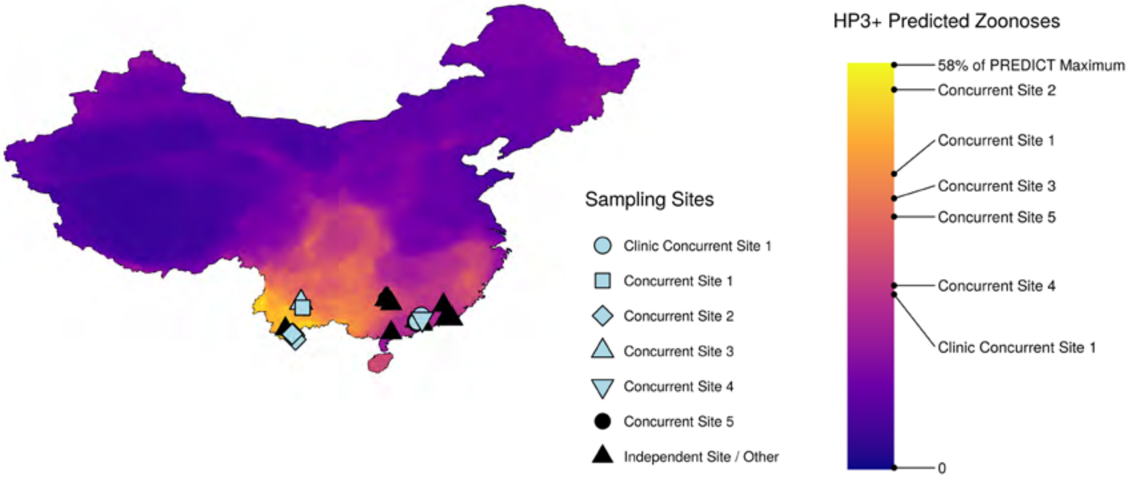


FIGURE 12. *Example of country-specific missing zoonoses map for China, including expanded predictions for all mammalian species and showing a hotspot for undetected viruses (lighter color) in Southwest China.*

WHERE DO CORONAVIRUSES LURK IN BATS? BAT-ORIGIN CORONAVIRUS RISK MAPS

Together with partners in China, we discovered Swine Acute Diarrheal Syndrome Coronavirus (SADS-CoV) that emerged from *Rhinopholus spp.* horseshoe bats to cause lethal outbreaks in pig farms in China (Zhou et al., 2018). The PREDICT team analyzed the overlap between all known bat reservoirs for this group of coronaviruses and regions in Asia where pigs are produced most intensively (Figure 13). These maps are now available to help target surveillance for mitigating future spillover events with relevance to predicting future SARS-related CoV emergence, as the same Rhinolophus bat hosts carry both groups of viruses.

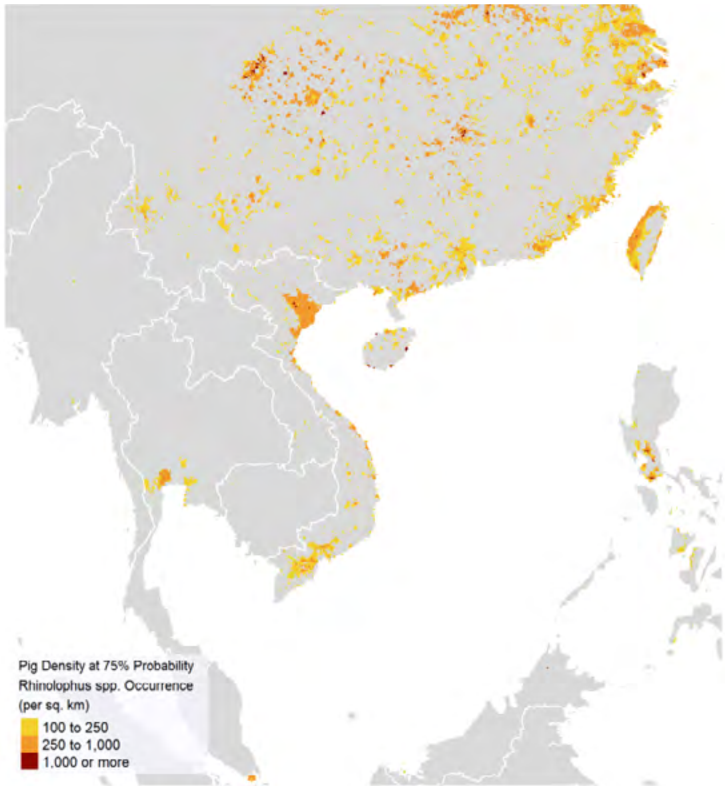


FIGURE 13. *Areas of greatest risk for SADS-CoV and SARS-CoV spillover from bats to swine. Based on species distribution models for three* **Rhinolophus spp.** *host species where probability of occurrence is high (>75%) and there is overlap with pig densities that are indicative of intensive pig farming (>100 heads per km2).*

WILL CLIMATE CHANGE AFFECT THE DISTRIBUTION OF KNOWN VIRAL THREATS?

Nipah virus is a deadly bat-origin paramyxovirus that spills over to the human population due to a suite of ecological, socioeconomic, and demographic factors. PREDICT used ecological niche modeling to investigate the potential role that climate change could play in the future risk of Nipah virus infection and the risk of other henipavirus emergence. The results showed that climate change is most likely to lead to elevated risk from henipaviruses due to expanding host ranges, although in some specific areas the risk will remain relatively stable or even decline as a result of shrinking host ranges (Figure 14).

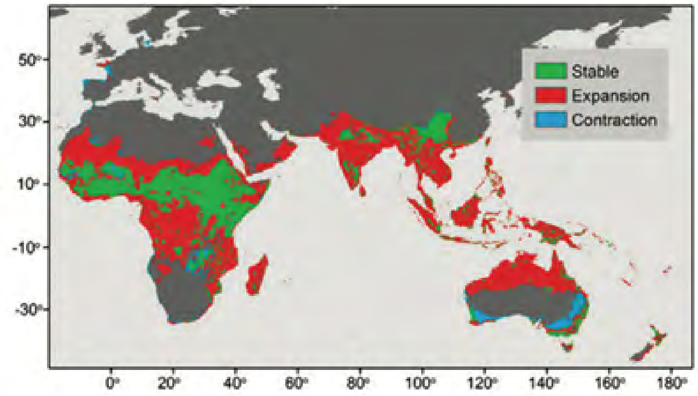


FIGURE 14. ***Climate change is likely to be a key factor and will expand and shift the future risk for henipaviruses emergence in Asia and Africa. This finding has important implications for managing public health risks and understanding drivers of disease emergence into the future (Daszak et al., 2013, PNAS).***

CAN WE PREDICT THE HUMAN-MEDIATED SPREAD OF EMERGING DISEASES?

Human movement patterns can predict the spread of human-to-human transmitted viruses and can inform mitigation measures, including heightened surveillance and staging of outbreak response personnel and supplies. The PREDICT project supported the development of the flight risk tracker (FLIRT) app that uses airline data and a model of passenger movement to forecast disease spread for human transmitted diseases (Huff et al., 2016). The app was used to map flights out of Wuhan, China in mid-January 2019, and map potential cities for early spread of COVID-19 (Figure 15). This predictive tool correctly showed Bangkok as a highly likely destination for passengers out of Wuhan during the same week that the PREDICT Thailand team supported the Ministry of Public Health to identify the first imported case of COVID-19 on Jan 13th, 2020 - the first documented instance of international spread.

> *The FLIRT app was used to map flights out of Wuhan, China in mid-January 2019, and map potential cities for early spread of COVID-19.*

WUH × WHZ × IUO ×

☑ Include airports within 50 miles

Start 01/17/20

End 01/31/20

Results Loaded: 529 Total: 529

Level: 1
Code: SFO
Name: San Francisco Intl
City: San Francisco
Country: United States
Region: North America
Normalized: 0%
Total Seats: 0

FIGURE 15. ***A screenshot from the Flight Risk Tracker (FLIRT) app, which shows flights out of Wuhan, China in mid-January 2019, along with the potential cities for early spread of COVID-19.***

PREDICT also used airline travel data to run 'what-if' scenarios and predict the spread of multiple other high-consequence EIDs over the last decade (Figure 16). These included Influenza A/H7N9 emerging from China, MERS from Saudi Arabia, and Ebola virus from West Africa. These maps have identified the hypothesized most at-risk countries for each virus, with good concurrence to the subsequent spread of SARS-CoV-2.

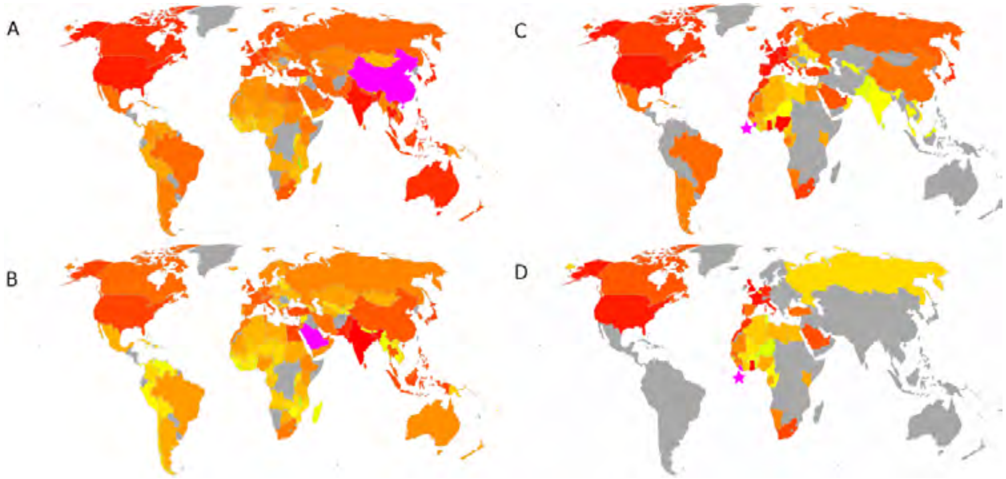


FIGURE 16. ***What-if scenarios to predict geographic spread for (A) Influenza A/H7N9 emerging from China, (B) MERS from Saudi Arabia, (C) Ebola virus from Sierra Leone, and (D) Ebola from Liberia. PURPLE = country of origin in modeled scenarios. Countries in RED = earliest arrival; YELLOW = intermediate; GREEN = latest arrival; GREY = countries that cannot be reached from origin without more than two legs of air travel.***

SARS, COVID-19 & DISEASE X

Coronaviruses have spilled over from bats to people or livestock leading to four significant outbreaks of novel diseases in the last 20 years: SARS-CoV, MERS-CoV, SARS-CoV-2, and SADS-CoV (see *Chapter 3*). Three of these outbreaks emerged first in China, and this group of viruses represents a major and continued threat to human health and food security as the next potential Disease X. We used our discovery of alpha- and betacoronaviruses to analyze the relationships among SARS-CoV, SARS-CoV-2, and other bat- and pangolin-CoVs (Latinne et al., 2020). We showed that the pangolin-CoVs are not likely the progenitors of the SARS-CoV-2 clade, but that the virus evolved from a group of bat-CoVs, and its progenitor is yet to be discovered (Figure 17).

We then reconstructed the bat hosts and locations (provinces) in China most likely to be the source of host-jumps for alpha- and betacoronaviruses using PREDICT data (Figure 18). We found that Vespertilionid and Rhinolophid bats are the evolutionary sources of alphacoronaviruses, while Rhinolophidae, Vespertilionidae, and Pteropodidae are the evolutionary sources of betacoronaviruses. We also discovered that Southwestern and Southern China are evolutionary hotspots of alpha- and betacoronaviruses, respectively.

> *Southwest and Southern China are evolutionary hot spots for bat coronaviruses.*

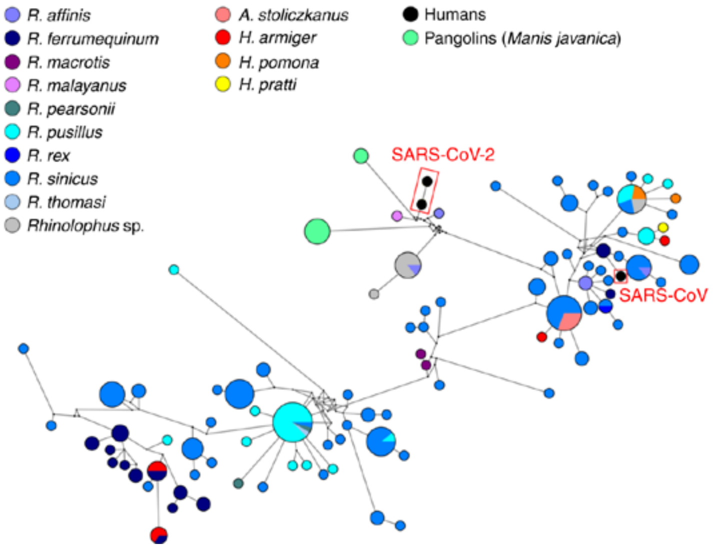


FIGURE 17. ***Phylogenetic network analysis showing the diversity of SARS-related CoVs found from bats and pangolins (bright green) in China.***

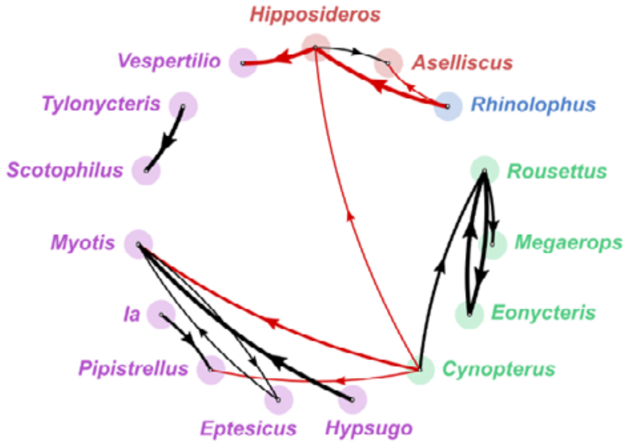


FIGURE 18. ***Evolutionary origins and cross-species transmission of bat coronaviruses in China. Analysis shows that the bat genera Rhinolophus is one of the most important source of strain diversity in beta-coronaviruses, the group that includes SARS-CoV-2, SARS-CoV, and MERS. Red lines correspond to host switches among bat genera belonging to different families, black lines correspond to host switches among bat genera from the same family. Arrows indicate the direction of historical virus host jumping.***

WHAT SPECIES HAVE THE HIGHEST POTENTIAL FOR HARBORING THE NEXT EMERGING VIRUS?

We developed a new strategy to identify sites on the planet that likely have the highest number of unknown high-risk viruses that are yet-to-be-discovered (“missing zoonoses”). In a paper published in *Nature* (Olival et al., 2017), we showed that the number of viruses and zoonoses a given mammal species can harbor is predictable, and that bats, rodents and primates together harbor the majority of as-of-yet identified viruses. The genetic relatedness of two host species, their geographic overlap, and other traits like body mass determine the zoonotic virus richness for each mammal species.

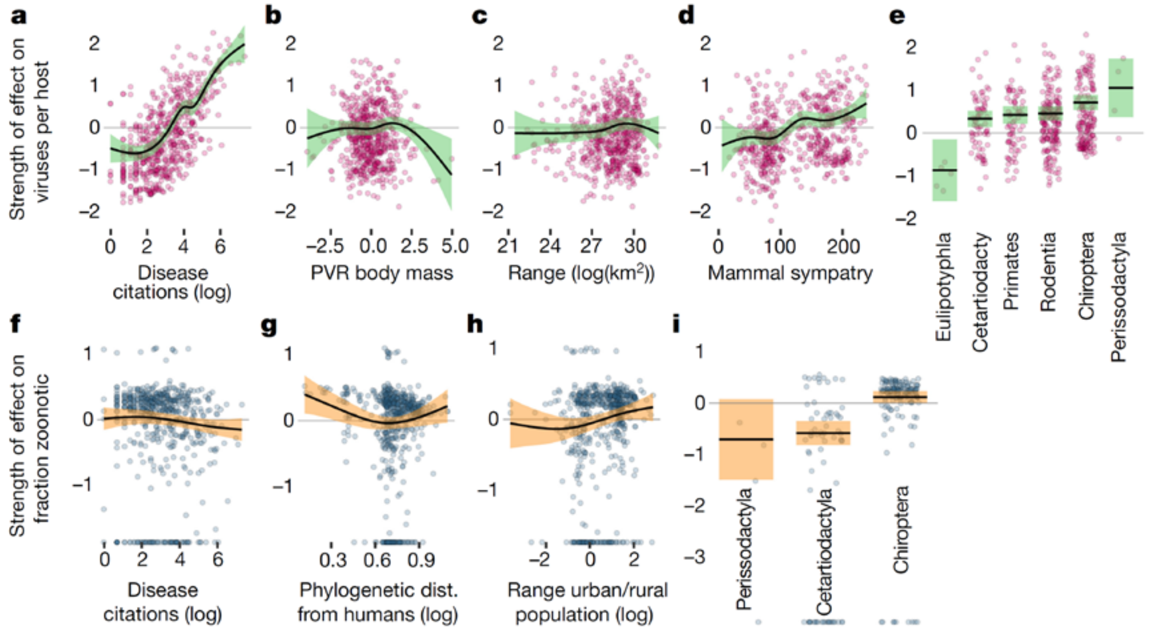


FIGURE 19. ***Predictors of total viral richness (top row) and zoonotic virus richness (bottom row) for mammal species (from Olival et al. 2017). Plots on far right (e and i) show which groups of mammals harbor a greater diversity of viruses (above line) then would be predicted using the other species-specific traits. These models were used to construct ‘missing zoonoses’ and ‘missing viruses’ maps to improve cost-effectiveness of virus discovery efforts (see Figure 11).***

WHAT VIRUSES WILL EMERGE & HOW MANY?

Our analyses showed that the factors determining which viruses are likely to emerge are predictable. "Some of these factors are virus-specific traits that can be deduced once a virus is characterized, such as having single-stranded RNA and being more prone to rapid evolution, or the ability to recombine or replicate in the cytoplasm of a host cell. However, we also showed that virus-specific factors, such as how widely divergent the taxonomy of the hosts that a virus infects is (its host plasticity or host breadth), are useful predictors of the ability of a virus to jump from one species to another, and therefore threaten human health. We used these traits with ecological and epidemiological risk factors for emergence to develop ranking algorithms to prioritize which of the newly discovered viruses are important enough for further characterization (Figure 20). The PREDICT SpillOver tool was developed to combine all known virus- specific and virus-independent risk factors to rank all viruses discovered during the PREDICT project as well as from external researchers (see *Chapter 8*).

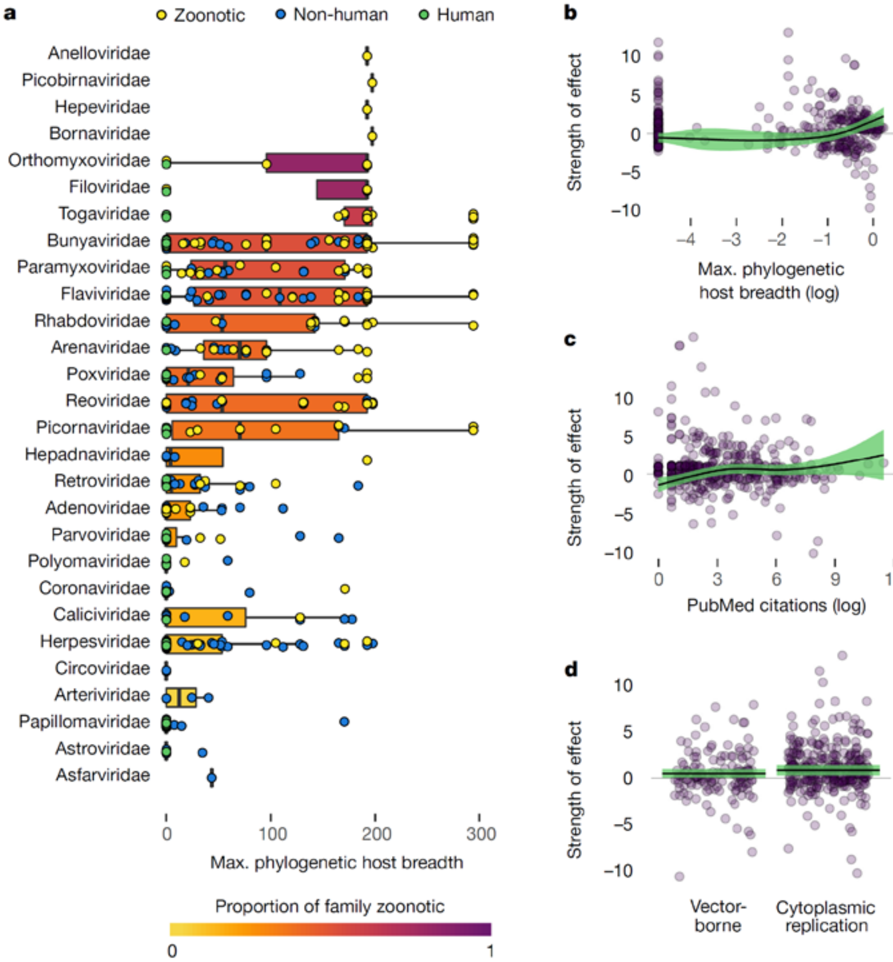


FIGURE 20. ***Ranking of all mammalian viruses known prior to the start of PREDICT by phylogenetic host breadth (a), a risk factor for zoonotic disease potential that measures the genetic distance between known hosts. Also showing other virus-specific predictors of zoonotic potential (b-d) from Olival et al., 2017. This analysis was incorporated into the SpillOver tool to rank all PREDICT-discovered viruses for their potential to spillover.***

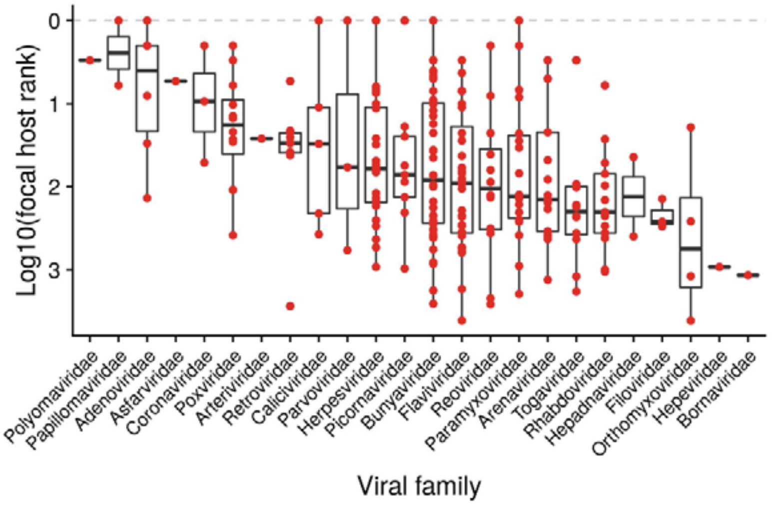


FIGURE 21. ***Ability to predict a virus' reservoir hosts varies across virus families. Families are ordered along the x axis in order of decreasing predictability (to the right). From Albery et al., 2020.***

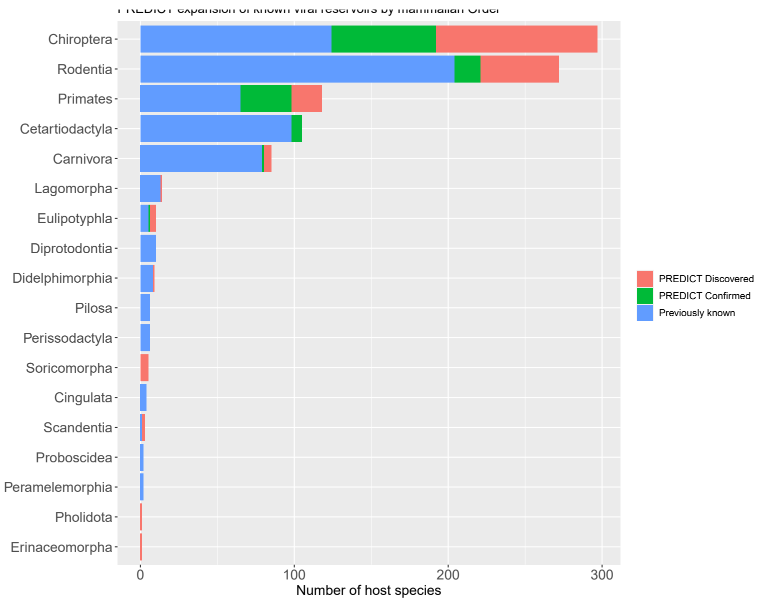


FIGURE 22. ***Expansion of known mammal host species for the PREDICT project's high priority virus families. Number of mammal species known to be virus reservoirs shown for each taxonomic order, with stacked bar charts showing number of pre-PREDICT identified hosts (blue), PREDICT confirmed host species (green), and newly discovered host species by PREDICT sampling and testing efforts (red).***

Using the phylogeographic model (based on species geographic overlap and genetic relatedness) and network analysis approach of Albery et al., 2020, we developed a tool to identify probable mammalian hosts for any given virus. This model can predict observed host species for 250 viruses with at least two known (non-human) mammal hosts. These species-level rankings can be used to set sampling priorities for public health efforts seeking to identify hosts of a novel zoonotic virus, where one or more hosts are already known. Across all 250 viruses, the median ranking of the left-out host was 72 out of a potential 4196 mammals (i.e. in the top 1.7% of potential hosts), but ability to predict a virus' hosts varied by virus family (Figure 21).

EXPANDING OUR KNOWLEDGE OF VIRUS HOST RANGE

Through the PREDICT project's extensive sampling of mammal species throughout Asia, Africa, and the Middle East, we greatly expanded our knowledge of host range for key viruses and virus families. We identified viruses in 196 species of mammals that were previously unknown to be virus reservoirs, expanding the number of hosts for key virus groups by over 30% from the pre-PREDICT baseline of information that took 70+ years to accumulate (Olival et al., 2017).

We also confirmed the presence of viruses in another 127 species of mammals that have been previously studied, in most cases discovering viruses new to science (Figure 22).

WHERE ARE THE MOST EFFICIENT PLACES FOR VIRUS DISCOVERY?

To estimate the costs and optimize potential sampling sites around the world for maximal virus discovery, we used spatial analyses combining data on mammal and waterbird biodiversity, hotspot risk, 'missing zoonoses', and access costs. We trialed this approach for the Global Virome Project (Carrol et al., 2018) (see *Chapter 8*), and identified select sites where a global sampling strategy would be most cost-efficient for identifying the maximum number of unique viruses. We also refined maps to prioritize future virus discovery at a national level for each PREDICT country (Figure 23). These analyses were then run independently for each PREDICT country to support planning for National Virome Projects.

HOW MANY ANIMALS DO YOU NEED TO SAMPLE TO DETECT ALL POTENTIALLY DEADLY VIRUSES?

Using our data, we developed an interactive viral accumulation curve tool and searchable table to allow in-country staff to explore virus discovery data and compare the efficacy of different sampling strategies and near real-time adjustment of sampling priorities to maximize virus discovery (Figure 24). These curves inform on how many individual animals should be sampled to reach saturation in virus discovery, providing a level of confidence that sufficient sampling was done to detect all of the potentially dangerous viruses in a species.

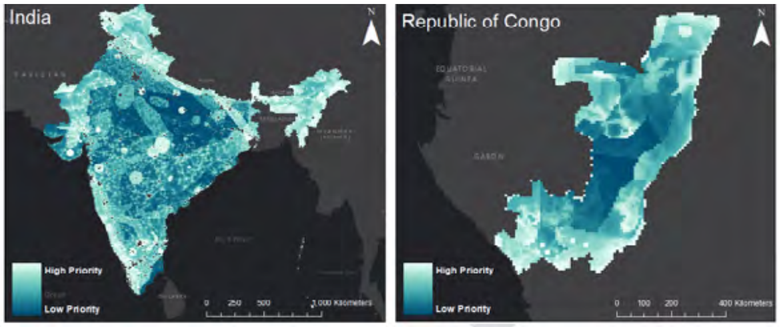


FIGURE 23. ***Example of prioritization maps for India and Republic of Congo showing the most cost-effective sites (lighter color areas) to maximize future virus discovery from wildlife.***

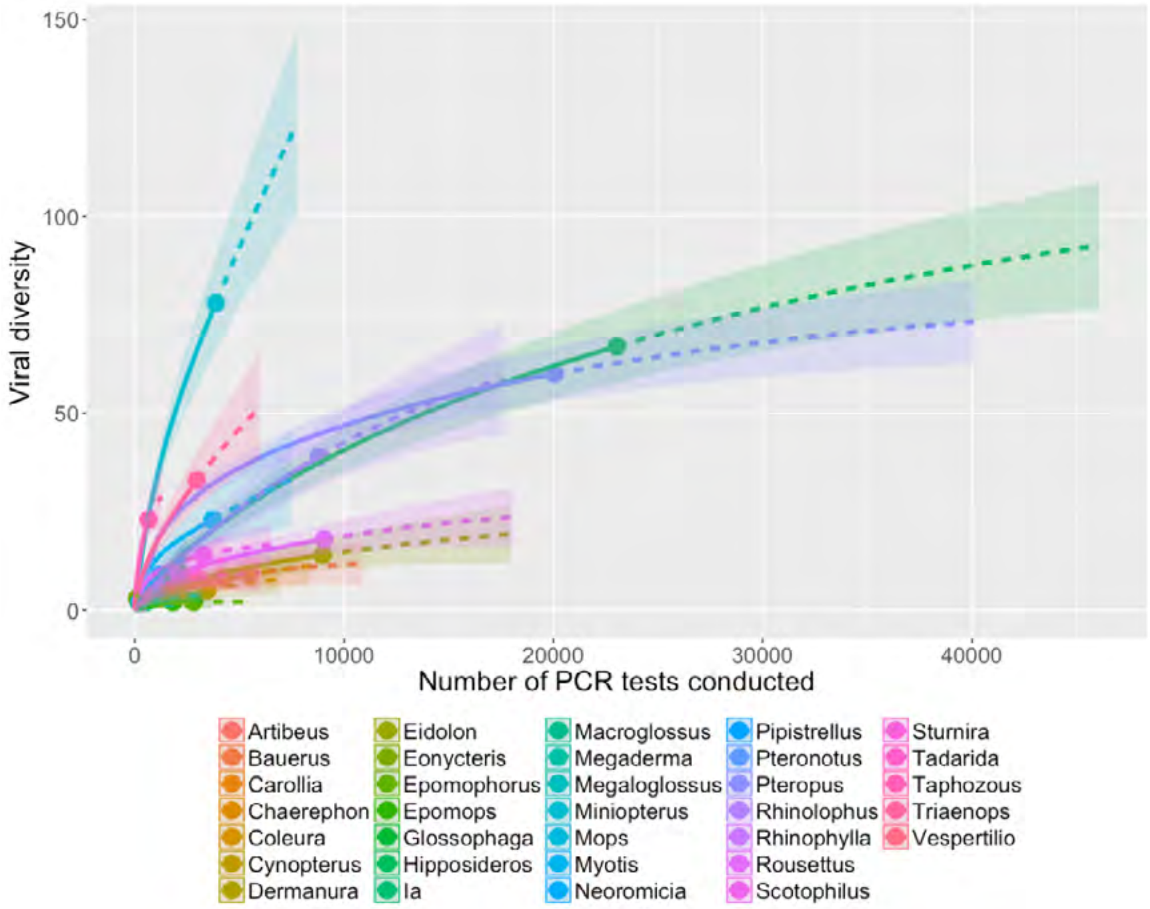


FIGURE 24. ***Viral accumulation curves for select mammal genera sampled under PREDICT. These analyses were built in to an interactive tool for PREDICT teams to explore virus discovery data and adjust sampling targets and protocols as needed to maximize virus discovery.***

WHEN IS RISK OF EMERGENCE HIGHEST?

> *A single intervention could reduce spillover risk for multiple viruses.*

We conducted analyses to examine whether surveillance can be targeted seasonally based on when the risk of disease emergence is greatest. We used longitudinal serological data from Bangladesh to identify co-circulation dynamics of Nipah, filoviruses, and *Rubulavirus* in a population of *Pteropus medius* bats (Figure 25). We analyzed these data to show that individual bats can be co-infected with multiple viruses. Interventions to mitigate the spillover of viruses from this one fruit bat species need to consider different viruses and their periods of shedding. Thus, a single intervention to mitigate human exposure may have the benefit of reducing spillover risk for multiple viruses.

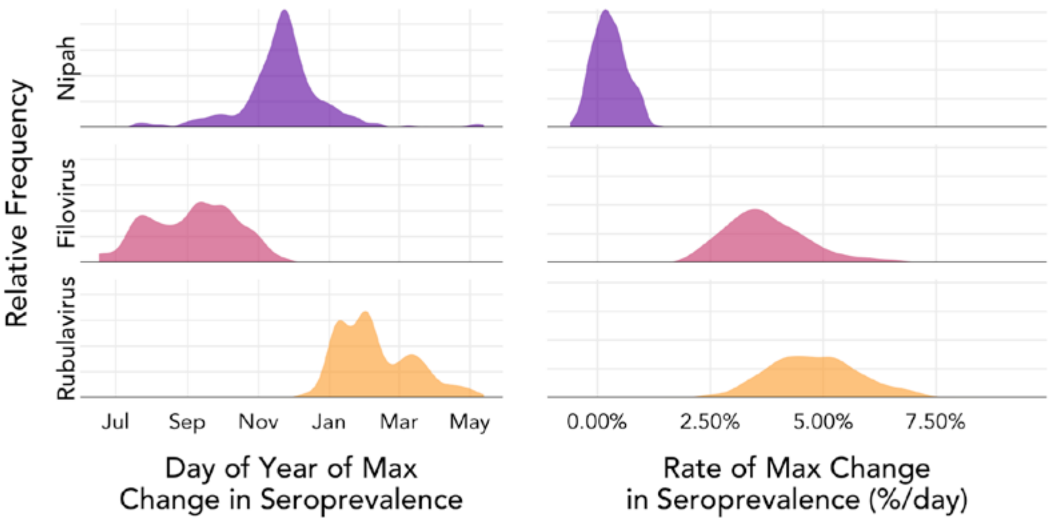


FIGURE 25. ***Analysis of serological data for three viruses to determine the timing and strength of antibody circulation in juvenile bats from a longitudinal dataset from Bangladesh. Plots show periods of the year when the increase in seroprevalence is greatest (left) and the rate of change for each (right). Plots display the relative frequency from 1,000 generalized additive model runs.***

We also developed a model to test for seasonal patterns in bat viral shedding while accounting for other potentially important factors (e.g. age, gender, reproductive status) and controlling for methodological and technical variation within the data (Figure 26). This analysis shows that bats are less likely to be actively shedding virus during periods of pregnancy. This analysis uses a hierarchical Bayesian model and demonstrates the value of big datasets to the global science community to understand risk factors for spillover and periods of the greatest value for sampling wildlife to identify viruses.

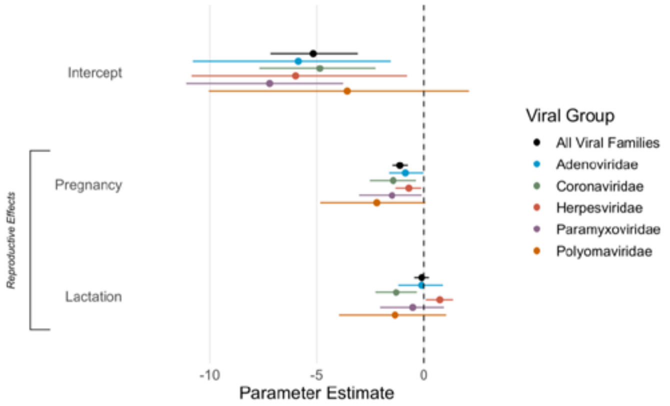


FIGURE 26. ***Modeled predictions of viral shedding against bat reproductive status and viral family. This shows that, in general, pregnant bats have a lower predicted viral prevalence than non-pregnant bats (model parameter estimate below 0 = lower viral shedding).***

WHAT ARE THE LONG-TERM ECONOMIC DAMAGES OF PANDEMICS?

To assess long-term trends in pandemic risk, we calculated expected global economic damages from EIDs over the next 50 years (Figure 27) using data from the Emerging Infectious Disease Repository (EIDR). We estimated that, if no coordinated global action is taken to reduce pandemic risk, a total of $3.6 trillion of economic damages will occur. Investing in a disease reduction program that reduces the number of events or size of events by only 5% gives a 45:1 return-on-investment. We also conducted economic analysis trainings in partnership with One Health Network to help design an economic model of land conversion and to support economic modeling that is locally relevant to stakeholders.

HOW MUCH WILL IT COST TO PREVENT PANDEMICS?

In 2020 during the COVID-19 pandemic, we contributed to an updated economic analysis to quantify the benefits of pandemic prevention measures for both public health and for the environment (Dobson et al., 2020). This study showed that pandemic mitigation measures, such as direct forest protection payments could achieve a 40% reduction in risk, and that the total present value of pandemic prevention costs for 10 years (including PREDICT) would only equal about 2% of the global costs of the COVID-19 pandemic. These findings highlight the importance of implementing global strategies for pandemic prevention as soon as possible in order to avoid soaring costs and economic damages.

> *Pandemic mitigation measures, such as direct forest protection payments could achieve a 40% reduction in risk.*

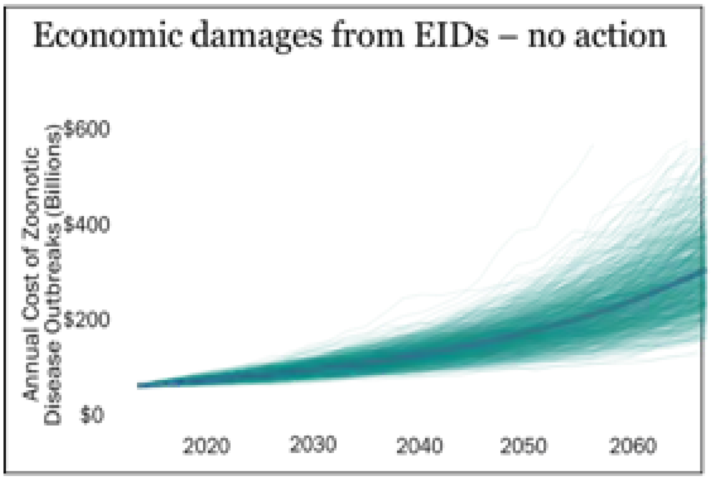


FIGURE 27. Economic damages from EIDs

> *Pandemic prevention costs for 10 years (including PREDICT) would only equal about 2% of the global costs of the COVID-19 pandemic.*

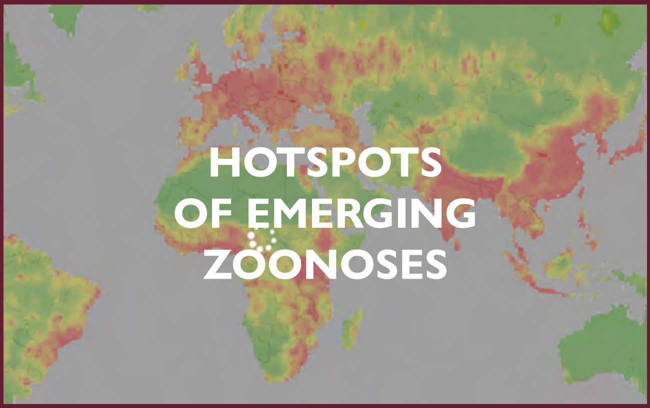
ADDITIONAL PRODUCTS & INSIGHTS

PREDICT's cross-cutting Modeling & Analytics team worked collaboratively with our surveillance, behavioral risk, laboratory, and information management teams to develop cutting-edge tools to better understand disease emergence and spread, refine on-the-ground virus discovery efforts, and identify potential intervention strategies at high-risk animal-human interfaces. Ultimately, all of the models developed by the team helped improve evidence-based strategies and One Health policy recommendations to minimize pandemic risk. Select analyses are available as Emerging Disease Insights, maps, and models at: **https://p2.predict.global/insights**

How do we determine which viruses pose the greatest threats to humans? PREDICT scientists developed a new tool for ranking the risk of viral spillover from animals to humans.

Our scientists sampled straw-coloured fruit bats in Ghana and Tanzania over the course of a year to learn about the peak season for shedding viruses and to identify the best times of year for disease control.

PREDICT discovered Bombali ebolavirus in bats in West Africa. But where do these bats live and what areas may be at highest risk of spillover?



Where will the next viral threat emerge?

GLOBAL COST OF EMERGING INFECTIOUS DISEASES

We make the economic case for investing in large-scale disease surveillance and prevention programs such as the Global Virome Project.

ENHANCING COLLABORATION ON EMERGING INFECTIOUS DISEASES RESEARCH

To counter the significant global health threat that emerging infectious diseases represent, China and the US have been leading efforts in preparedness with unparalleled resources, widespread engagement, and national and geopolitical imperatives to contribute to global health security.

UNDERSTANDING CROSS-SPECIES TRANSMISSION OF BAT CORONAVIRUSES IN CHINA

How did SARS-CoV-2 emerge? Through the PREDICT project's work in China we identified hotspots for coronavirus (CoV) emergence and detected two CoV sequences in bats which are the closest known relatives of the virus causing COVID-19.

SIMULATION: NOVEL BAT CORONAVIRUS FROM GUANO HARVEST

What would happen if a new virus discovered through the PREDICT project spilled over into humans? We simulate the emergence and spread of a newly detected coronavirus from bat guano to people and provide recommendations for disease control to prevent a pandemic.

IDENTIFYING THE NEXT ZIKA

The pandemic spread of Zika emphasized the need to better understand the distribution, host range, and epidemic potential of other understudied flaviviruses. We explore both current and potential risks of flavivirus spillover and spread.

IMPROVING VIRAL SAMPLING & DISCOVERY

We highlight our innovative analytical tool that answers the question: how many samples do we need to collect and test to find the majority of viruses in an animal host?

PHOTO: PREDICT NEPAL

TOWARDS A BRIGHTER & HEALTHIER FUTURE

Over the last decade, the PREDICT project has built a deep understanding of which geographies, species, animal-human interfaces, and behaviors represent a particularly high-risk for future spillover events. We have made unparalleled progress in developing the technological tools and cross-disciplinary approaches needed to forecast future emerging diseases, recognizing that those tools will evolve and become more precise over time. We have showed through economic analyses and cost-benefit approaches that emerging disease prevention is feasible, and much more affordable than the alternative of waiting for diseases to emerge and then trying to control them afterwards.

But we envision a brighter future, one where One Health surveillance networks continue to expand around the world under national ownership and leadership. Here, multiple laboratories in each country, particularly in EID Hotspots around the world, have the capacity to detect and characterize the rich diversity of wildlife viruses poised to spillover but that currently remain undiscovered. In this future, data from surveillance and discovery efforts are collected in near-real time and shared with in-country modeling and analytical teams, and also transparently with international partners, to refine surveillance and ultimately to develop effective early warning systems for disease spillover. We envision a world that builds off the legacy of the PREDICT project and continues to strengthen evidence-based approaches to preventing emerging zoonotic pathogens and stopping the next pandemic in its tracks.

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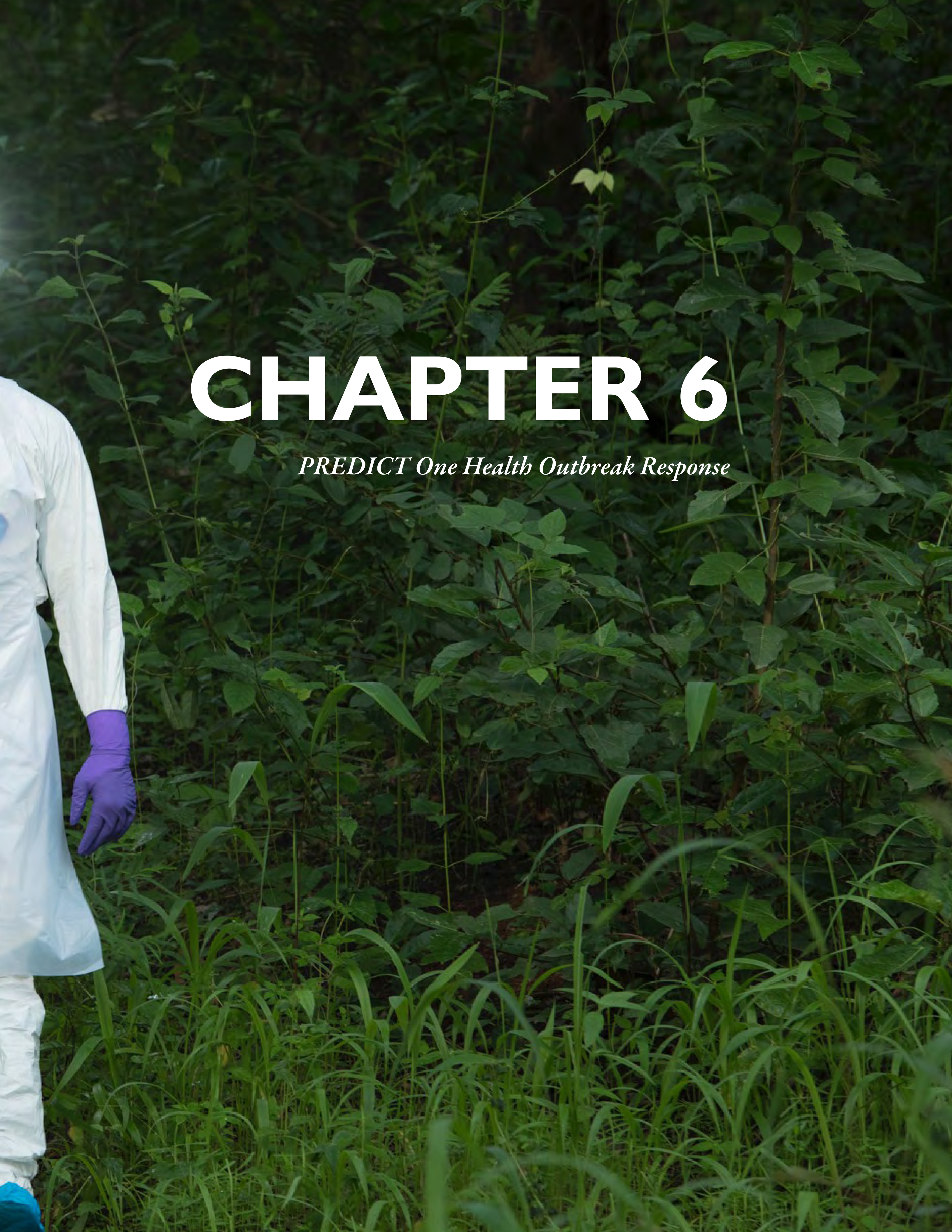
PHOTO: SIMON TOWNSLEY



CHAPTER 6

PREDICT One Health Outbreak Response

KEY TAKEAWAYS

- The PREDICT project provided critical outbreak support to governments and international organizations during 53 outbreaks affecting humans, domestic animals, and wildlife, in 16 countries.
- Ebola virus disease and viral hemorrhagic fevers were the most common outbreaks for which PREDICT provided assistance.
- We supported the Democratic Republic of the Congo during six Ebola virus disease outbreaks between 2012 and 2019.
- We incorporated the One Health approach in providing outbreak assistance through field visits, sampling, diagnostic support, personal protective equipment provision, and surveillance.
- The PREDICT project team evaluated the reporting of Salzburg metrics for One Health surveillance across PREDICT reports on outbreak or health events for which PREDICT assisted in investigation or response.
- We conducted an outbreak simulation workshop in Tanzania, the outbreak training of which was made available to other countries.
- Our team developed a modeling framework using satellite and high-resolution population data to build a network for simulating infectious diseases, helping policy makers and outbreak responders determine outbreak spread and decide areas for targeted control.
- Our PREDICT-trained network across 26 countries jumped into action for coronavirus detection and outbreak response during the SARS-CoV-2 pandemic, including early detection of the first COVID-19 cases before a specific assay targeting the novel coronavirus was available.

It was a normal day for staff at the health centre until an older woman from a nearby village arrived with a high fever, gastrointestinal signs, and muscle pain. She was admitted to the hospital, started treatment with supportive care and appeared to improve. But when the head clinician returned the next day, he learned the woman had passed away overnight. Clinic staff sent a messenger to the family, and they learned that two others in the woman's household were acutely ill. Within a week, the health centre began to receive unusually high volumes of febrile cases with non-specific symptoms; dozens of patients with fevers were admitted, and staff were rapidly overwhelmed. Initially they attributed this onslaught to the beginning of malaria season, until reports surfaced about sick animals: primates found dead in the forest and a "strange disease" in village cats. Was it an outbreak?

A ONE HEALTH APPROACH TO OUTBREAK RESPONSE

PREDICT Mongolia's team conducting a physical exam on a common shelduck at Sangiin Dalai Lake in response to reports of a wild bird die-off.

PHOTO: PREDICT MONGOLIA

Between 2010 and 2019, PREDICT provided critical support to governments and international organizations during 53 outbreaks in 16 countries (Figure 1), in addition to COVID-19 response. Ebola virus disease and viral hemorrhagic fevers were the most common outbreaks for which PREDICT provided assistance (26%), followed by animal/bird die-offs (15%) and influenza (9%). The vast majority of these outbreaks impacted human populations (66%) and non-human primates (17%), while animals such as bats, wild birds, domestic animals and poultry were also affected in other outbreaks (Figure 2).

With many outbreaks consisting of spillovers from animal populations to humans, PREDICT's outbreak response strategy incorporated the One Health approach in providing critical assistance in disease investigations and response assistance. PREDICT helped tackle outbreaks affecting human, domestic animal, and wildlife populations at their source (Figure 3) by providing diagnostic support, materials, and personal protective equipment (PPE), as well as conducting field visits, sampling, and surveillance.

> ***PREDICT provided critical support to governments & international organizations during 53 outbreaks in 16 countries.***

EBOLA VIRUS DISEASE, DEMOCRATIC REPUBLIC OF THE CONGO

PREDICT supported the Democratic Republic of the Congo (DRC) in EVD response activities spanning six outbreaks between 2012 and 2019. Of note are the following two EVD outbreak activities in the DRC's Equateur Province:

In August 2014, the DRC's Institut National de Recherche Biomédicale (INRB) formally requested the PREDICT laboratory to test samples from suspected cases of viral hemorrhagic fever for filoviruses using the PREDICT protocols. On August 23, PREDICT reported to the Deputy Director of INRB and CDC personnel in Kinshasa that several samples were found to be positive for Ebola virus. The following day, the government of DRC officially declared an outbreak of Ebola in the Equateur Province. PREDICT continued to support response efforts for the duration of the outbreak, which was declared over on November 15, primarily through laboratory diagnosis, sample collection, and provision of equipment and supplies.

Four years after the 2014 Ebola outbreak, the DRC's Ministry of Health again engaged PREDICT's support in responding to an Ebola outbreak in the Equateur Province, similarly requesting assistance with laboratory diagnostics at INRB. In addition to providing testing on patient specimens using their laboratory protocols, PREDICT provided PPE and sampling kits to the outbreak sites. The team also provided logistical support, such as freezer space to store Ebola vaccines, and trained an INRB field team member on animal sampling protocols.

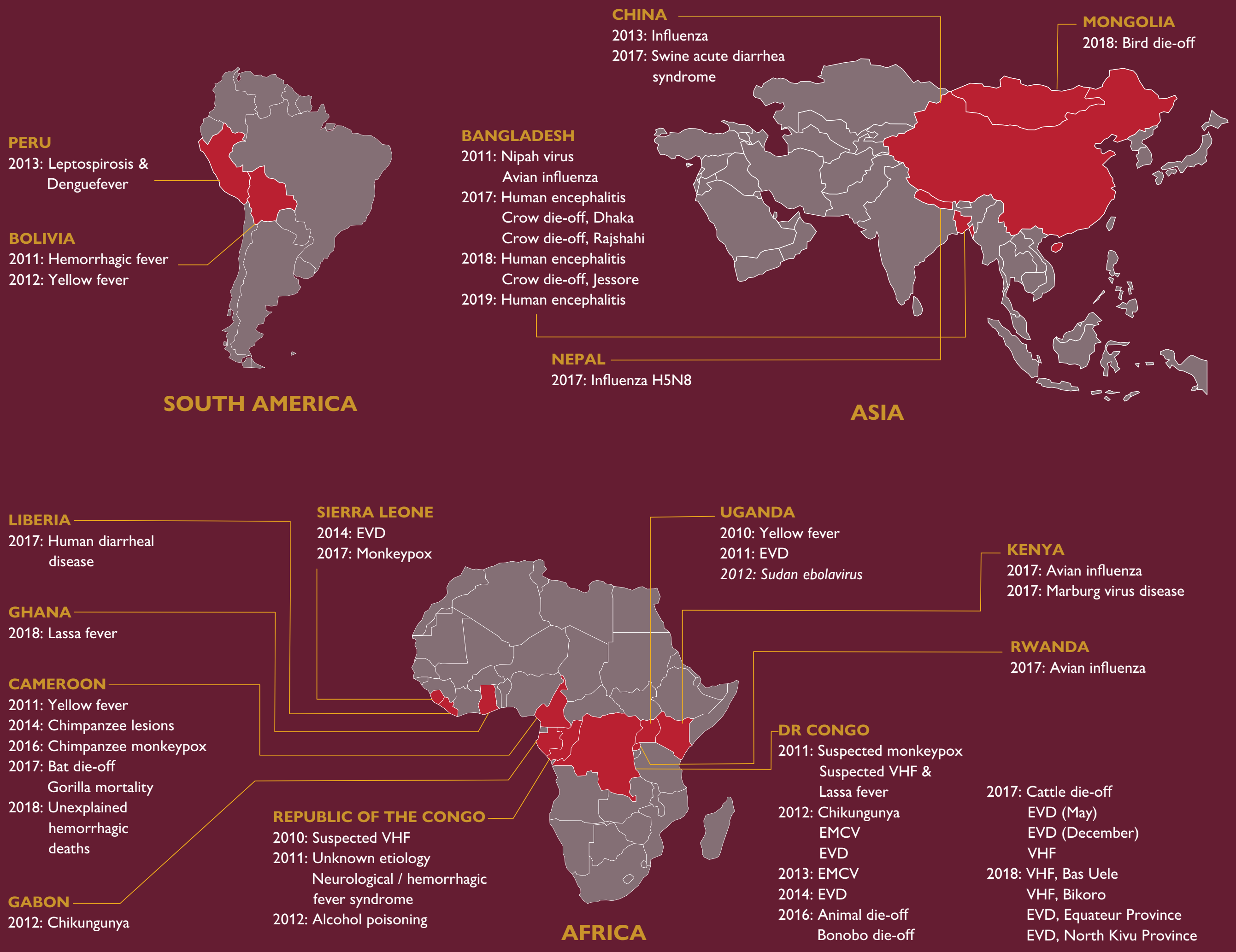
Ebola Host Project's Mohamed Turay untangles a bat from a mist net in Sierra Leone. PREDICT's Ebola Host Project, a regional effort across West Africa, worked to identify the animal host species of ebolaviruses.

PHOTO: SIMON TOWNSLEY

OPERATIONALIZING ONE HEALTH OUTBREAK RESPONSE HIGHLIGHTS

- **2016 (DRC)**: An animal die-off event in which over 400 domestic animals and a number of wild animals died with the same clinical symptoms prompted the Ministry of Agriculture to request investigative support from PREDICT, the Food and Agriculture Organization (FAO) and the Centers for Disease Control and Prevention (CDC). Five people who had reported eating meat from animals that had died or been sick also fell ill, suggesting possible pathogen transmission between animals and humans. In response to the outbreak, a PREDICT Field Ecologist collaborated with the team from the Institute for Conservation of Nature to collect specimens from sick and dead animals, which were tested according to PREDICT protocols for filoviruses, flaviviruses, bunyaviruses, and hantaviruses. Of the samples tested, five samples were successfully sequenced for hantavirus.
- **2017-2019 (Bangladesh)**: PREDICT teams responded to three separate crow die-off events across three districts of Bangladesh. Within days of being alerted to each of the die-off events, the Government of Bangladesh's Institute of Epidemiology, Disease Control and Research (IEDCR) requested PREDICT's support in the outbreak, predominantly through field visits and provision of technical assistance, laboratory assistance, and outbreak response training. In addition to the IEDCR, PREDICT team members collaborated on the outbreak surveillance and response with FAO, the Department of Livestock Services, and the One Health Secretariat, as well as various other institutions such as the Bangladesh Livestock Research Institute and the International Centre for Diarrheal Diseases Research, Bangladesh. The Government of Bangladesh confirmed laboratory results from two of the outbreak events, with specimens positive for H5N1 avian influenza. No signs or symptoms of human or domestic animal avian influenza infections were reported or observed during any of the three outbreak events.
- **2018 (Ghana)** Following a confirmed case of Lassa virus in a 26-year old male, the Government of Ghana's Wildlife Division of the Ministry of Land and Natural Resources requested PREDICT's support to provide technical advice and assistance for a field ecology investigation. PREDICT team members held refresher trainings for staff on biosafety protocols and PPE use, collaborated to provide community sensitizations, and conducted rodent capture and specimen collection. The investigation involved a collaborative response from the Ghana Health Service, the Noguchi Memorial Institute for Medical Research, and the School of Public Health at the University of Ghana.
- **2018 (Mongolia)**: the Mongolian State Central Veterinary Laboratory (SCVL) contacted the PREDICT Ulaanbaatar team to request assistance in response to a wild water bird die-off discovered at a lake in the Gobi-Altai Province. The PREDICT avian influenza team, already in the field conducting routine surveillance, joined SCVL within hours of their request at the die-off site. PREDICT provided technical assistance before collecting wildlife samples and recording GPS coordinates and video footage of sick birds. The team remained in communication with the SCVL for the duration of the outbreak to coordinate surveillance efforts, as well as the Mongol Els National Park rangers to monitor for sick or dead birds. SCVL conducted laboratory testing using both in-house and PREDICT's laboratory protocols, with specimens testing negative for both avian influenza and Newcastle disease.

FIGURE 1. PREDICT OUTBREAK SUPPORT



PERU
2013: Leptospirosis &
Denguefever

BOLIVIA
2011: Hemorrhagic fever
2012: Yellow fever

CHINA
2013: Influenza
2017: Swine acute diarrhea
syndrome

MONGOLIA
2018: Bird die-off

BANGLADESH
2011: Nipah virus
Avian influenza
2017: Human encephalitis
Crow die-off, Dhaka
Crow die-off, Rajshahi
2018: Human encephalitis
Crow die-off, Jessore
2019: Human encephalitis

NEPAL
2017: Influenza H5N8

LIBERIA
2017: Human diarrheal
disease

SIERRA LEONE
2014: EVD
2017: Monkeypox

UGANDA
2010: Yellow fever
2011: EVD
2012: Sudan ebolavirus

KENYA
2017: Avian influenza
2017: Marburg virus disease

GHANA
2018: Lassa fever

RWANDA
2017: Avian influenza

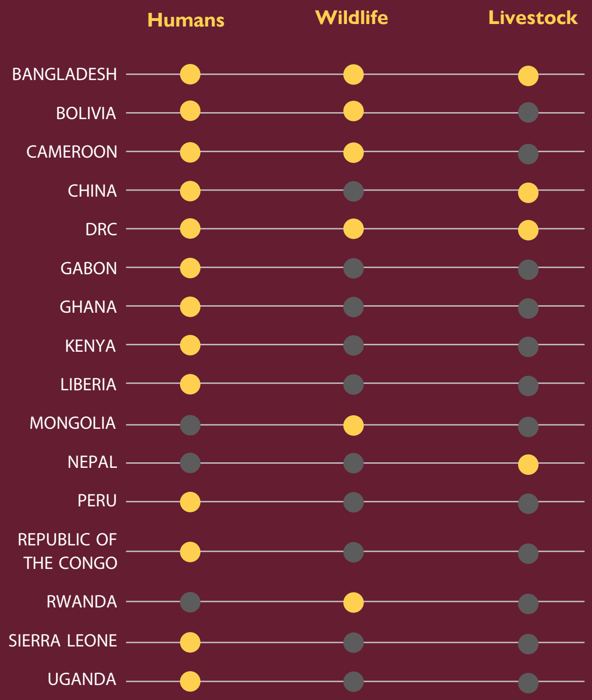
CAMEROON
2011: Yellow fever
2014: Chimpanzee lesions
2016: Chimpanzee monkeypox
2017: Bat die-off
Gorilla mortality
2018: Unexplained
hemorrhagic
deaths

DR CONGO
2011: Suspected monkeypox
Suspected VHF &
Lassa fever
2012: Chikungunya
EMCV
EVD
2013: EMCV
2014: EVD
2016: Animal die-off
Bonobo die-off
2017: Cattle die-off
EVD (May)
EVD (December)
VHF
2018: VHF, Bas Uele
VHF, Bikoro
EVD, Equateur Province
EVD, North Kivu Province

REPUBLIC OF THE CONGO
2010: Suspected VHF
2011: Unknown etiology
Neurological / hemorrhagic
fever syndrome
2012: Alcohol poisoning

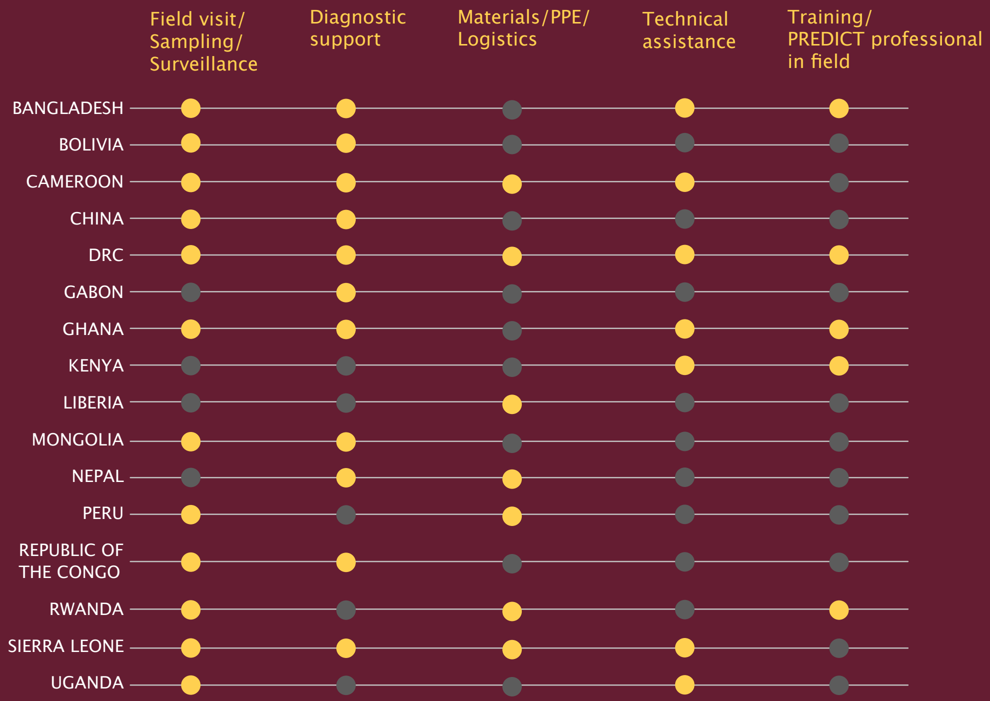
GABON
2012: Chikungunya

FIGURE 2. AFFECTED POPULATIONS



	Humans	Wildlife	Livestock
BANGLADESH	●	●	●
BOLIVIA	●	●	○
CAMEROON	●	●	○
CHINA	●	○	●
DRC	●	●	●
GABON	●	○	○
GHANA	●	○	○
KENYA	●	○	○
LIBERIA	●	○	○
MONGOLIA	○	●	○
NEPAL	○	○	●
PERU	●	○	○
REPUBLIC OF THE CONGO	●	○	○
RWANDA	○	●	○
SIERRA LEONE	●	○	○
UGANDA	●	○	○

FIGURE 3. OUTBREAK SUPPORT PROVIDED



	Field visit/ Sampling/ Surveillance	Diagnostic support	Materials/PPE/ Logistics	Technical assistance	Training/ PREDICT professional in field
BANGLADESH	●	●	○	●	●
BOLIVIA	●	●	○	○	○
CAMEROON	●	●	●	●	○
CHINA	●	●	○	○	○
DRC	●	●	●	●	●
GABON	○	●	○	○	○
GHANA	●	●	○	●	●
KENYA	○	○	○	●	●
LIBERIA	○	○	●	○	○
MONGOLIA	●	●	○	○	○
NEPAL	○	●	●	○	○
PERU	●	○	●	○	○
REPUBLIC OF THE CONGO	●	●	○	○	○
RWANDA	●	○	●	○	●
SIERRA LEONE	●	●	●	●	○
UGANDA	●	○	○	●	○

SARS-CoV-2 OUTBREAK RESPONSE

The PREDICT-trained network assisted 26 countries around the world in coronavirus detection and outbreak response by providing diagnostic support, technical assistance, and training, as well as funding for supplies and personal protective equipment (Figure 4). In the absence of an early specific assay targeting SARS-CoV-2, PREDICT's consensus-based PCR assay for coronaviruses was successfully optimized for the early detection of the virus in several countries in Asia.

COVID-19 SUPPORT HIGHLIGHTS

NEPAL

The first case of COVID-19 in Nepal was detected by PREDICT Nepal's implementing partner, the Center for Molecular Dynamics Nepal (CMDN), initially using our protocols in the absence of more specific assays at that time, and later confirmed by the WHO recommended Berlin Real Time PCR protocol. PREDICT provided training on the detection of SARS-CoV-2 to the National Public Health Laboratory (NPHL) and also supplied them with testing reagents and PPE. The PREDICT field team trained over 500 doctors, nurses, and frontline healthcare providers in proper use of PPE. Our team also worked closely with Nepal's Epidemiology and Disease Control Division (EDCD), Nepal Health Research Council (NHRC), and WHO in various COVID-19 related research and diagnostic activities, including whole genome sequencing of SARS-CoV-2 strains and community surveillance through environmental (sewage) sampling.

Working with a municipality hospital and the local government in the Kathmandu Valley, the PREDICT Nepal team helped set up a COVID-19 molecular testing laboratory in a community hospital: more than 3,000 people have been screened thus far for SARS-CoV-2 with PREDICT's support. Building on our experience with hospital and community-based surveillance of emerging diseases, we also launched Nepal's first community-based COVID-19 surveillance effort in one of the municipalities of the Kathmandu Valley. Additionally, our team was also involved in COVID-19 preparedness, surveillance and capacity building in the Mt. Everest region, a remote area of Nepal where where we helped establish a COVID-19 molecular testing facility.

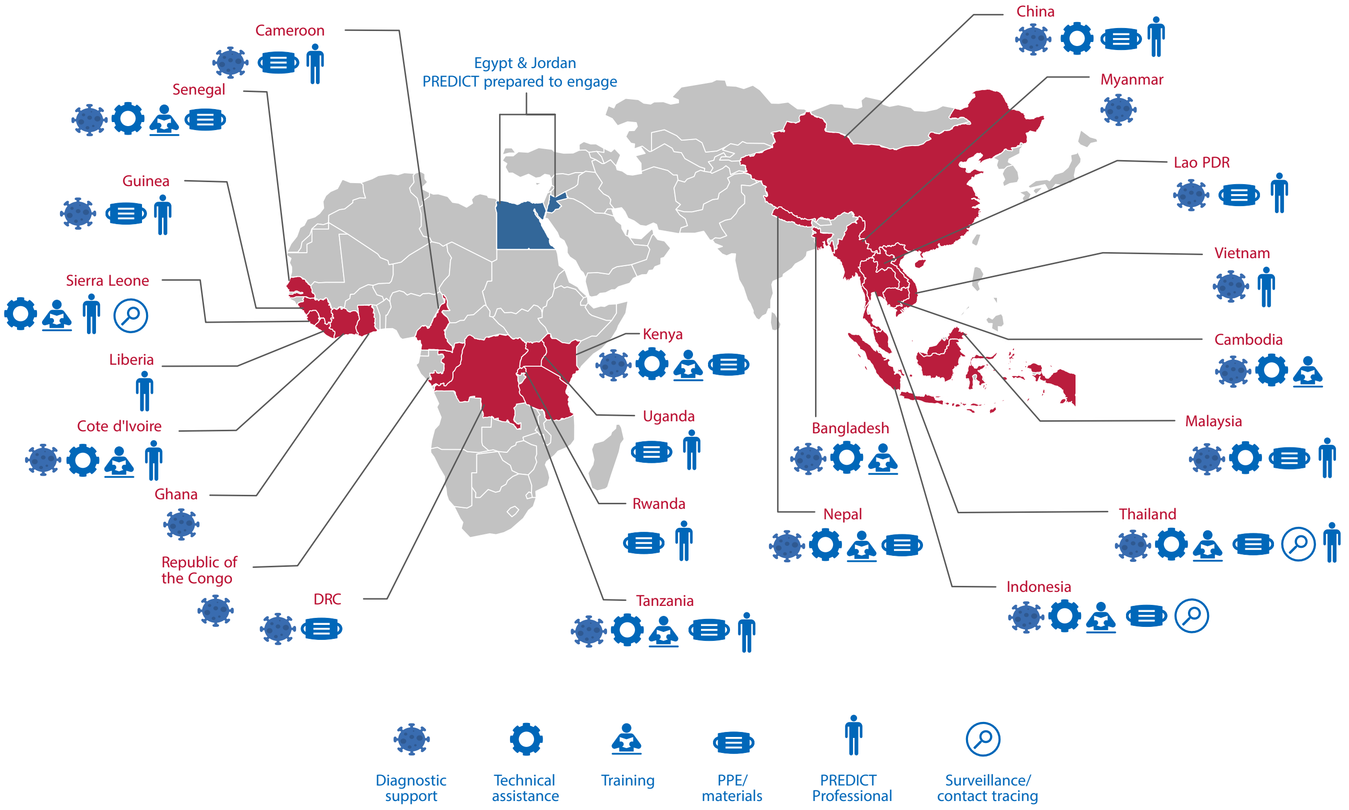
THAILAND

Our teams help test early cases suspected of COVID-19 in the country, and in consultation with the global team, ascertained which of our protocols detected SARS-CoV-2. We also designed a real time assay based on the short fragment once they sequenced the virus from a positive sample. Our team continued to test samples as requested by the Government of Thailand, and became one of the 50+ laboratories conducting COVID testing in-country while also working on sequencing and serological testing of samples.

SIERRA LEONE

In late March 2020, the President and Government of Sierra Leone issued a State of Emergency in an effort to contain the spread of SARS-CoV-2. By April 2020, PREDICT-trained Sierra Leonean professionals were providing technical assistance to the National Disaster and Health Emergencies Surveillance Pillar and Emergency Operations Center to support contact tracing of positive COVID-19 cases; administrative support to ensure contact tracers were actively following-up on reported contacts; and were being deployed to critical district surveillance sites (Kenema, Bo, Makeni, and Freetown). Our team worked in the field alongside first responders as contact tracers, community outreach specialists and data management personnel.

FIGURE 4. PREDICT SUPPORT DURING THE SARS-CoV-2 PANDEMIC

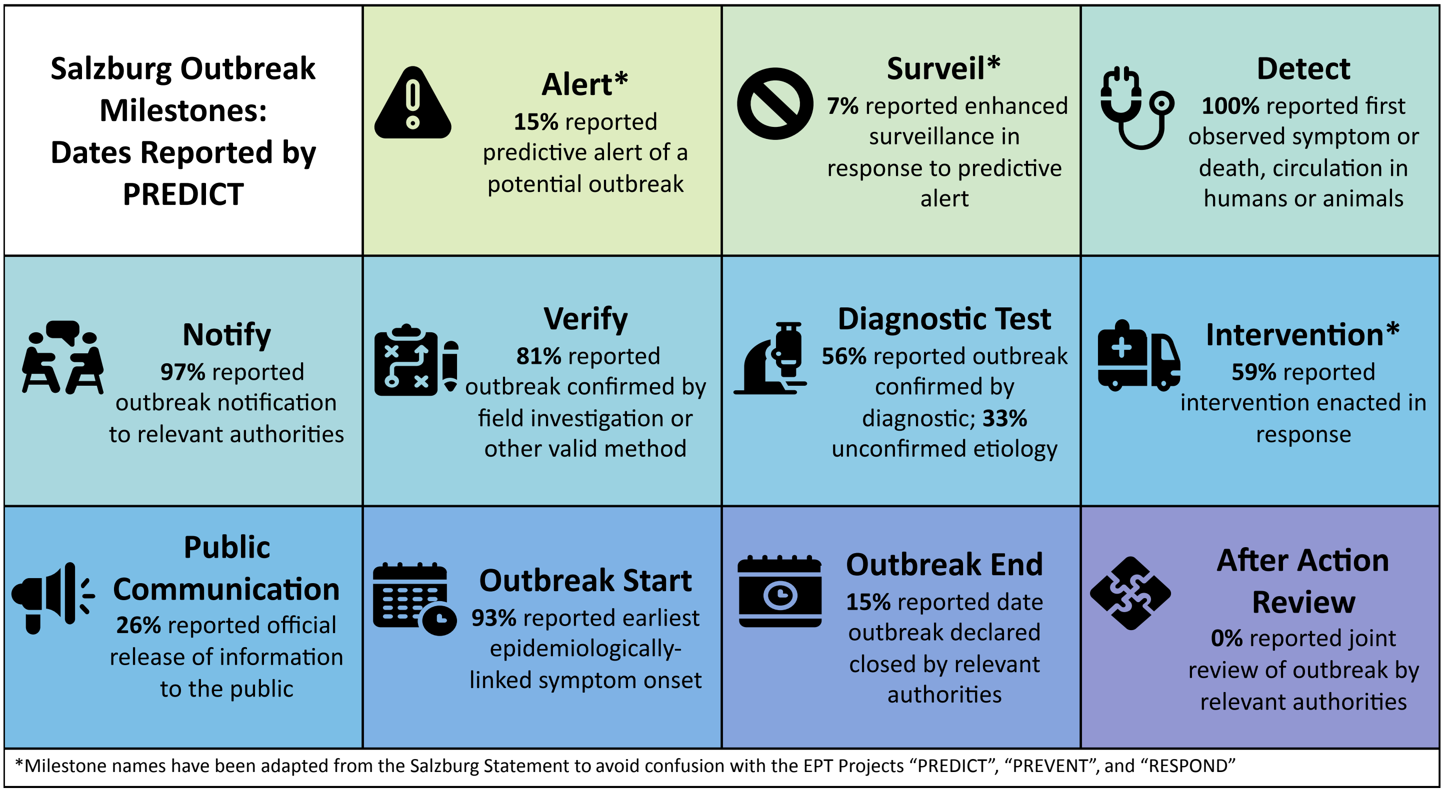


CONTRIBUTION TO SALZBURG METRICS FOR ONE HEALTH SURVEILLANCE

The COVID-19 pandemic has demonstrated mounting recognition worldwide of the urgent need for One Health surveillance practices. Coinciding with this pandemic, on May 4, 2020, the Salzburg Statement on Metrics for One Health Surveillance was released to the public, describing eleven key Outbreak Metrics. Defined as the time intervals between respective outbreak milestones (Figure 5), these metrics are designed to serve as standardized benchmarks that would allow countries to better understand their progress in finding, preventing, and responding to outbreaks across the animal, environmental, and human sectors. Metrics originally developed in 2018 by experts from the human health sector convened by the US-based non-profit Ending Pandemics and Salzburg Global Seminar were recognized to be excellent; however, there was still a clear need for a multi-sectoral One Health approach to epidemic preparedness. In a subsequent meeting in October 2019, experts from around the globe, including representatives from the PREDICT project, developed the current Timeliness Metrics, evolving from human health only to include animal and environmental health, as well.

Recognizing the call for One Health advocates to incorporate the Timeliness Metrics into outbreak activities and global workforce development training, we sought to evaluate our own reporting of these milestones in the program's standardized Outbreak or Health Event Rapid Reports. Toward this goal, team members evaluated the reporting of Timeliness Metrics across 27 Rapid Reports from distinct outbreak or health events for which the PREDICT project was invited to assist in the investigation or response (Figure 5). All 27 reports (100%) reported a specific date the outbreak was detected, with 97% reporting the date the relevant authorities were notified of the outbreak, 93% reporting the outbreak start date, and 81% reporting the specific date the outbreak was verified. Over half (56%) of the reports provided a specific date of the diagnostic or laboratory confirmation, with nine reports (33%) providing specific dates of laboratory testing with inconclusive results. Only four of the reports captured the date that the outbreak ended, with most of the Rapid Reports describing ongoing outbreaks.

Understanding that the milestones reported may vary given the outbreak, and that outbreak reporting has likely varied over the past decade, team members went further to conduct a scoping review to determine the frequency with which the Salzburg Statement outbreak metrics were reported in outbreak reports published in peer-reviewed literature or in outbreak reports published by major health agencies or organizations between 2010 and 2020. This scoping review includes an analysis of PREDICT Rapid Reports from all reported outbreak or health events.



Salzburg Outbreak Milestones: Dates Reported by PREDICT	**Alert*** **15%** reported predictive alert of a potential outbreak	**Surveil*** **7%** reported enhanced surveillance in response to predictive alert	**Detect** **100%** reported first observed symptom or death, circulation in humans or animals
Notify **97%** reported outbreak notification to relevant authorities	**Verify** **81%** reported outbreak confirmed by field investigation or other valid method	**Diagnostic Test** **56%** reported outbreak confirmed by diagnostic; **33%** unconfirmed etiology	**Intervention*** **59%** reported intervention enacted in response
Public Communication **26%** reported official release of information to the public	**Outbreak Start** **93%** reported earliest epidemiologically-linked symptom onset	**Outbreak End** **15%** reported date outbreak declared closed by relevant authorities	**After Action Review** **0%** reported joint review of outbreak by relevant authorities

*Milestone names have been adapted from the Salzburg Statement to avoid confusion with the EPT Projects "PREDICT", "PREVENT", and "RESPOND"

FIGURE 5. ***Frequency of Timeliness Metrics Outbreak Milestones reported across 27 Outbreak or Health Events Reports with direct PREDICT engagement.***

OUTBREAK WORKSHOP HIGHLIGHTS & MODELING TOOLS

OUTBREAK TRAINING EXERCISE, TANZANIA

The PREDICT team held an outbreak simulation workshop in Tanzania in 2017, focusing on a potentially zoonotic swine die-off scenario across multiple farms. This comprehensive workshop was held over a few weeks and involved cross-disciplinary participation with trainees from the Ifakara Health Institute and Sokoine University of Agriculture, representing both human and animal health professionals. After being piloted in Tanzania, this outbreak training was made available to other countries.

OUTBREAK MODELING TOOL USING SATELLITE IMAGERY & GIS DATA, RWANDA

Once spillover events and outbreaks have occurred, we stand an improved chance of mitigating and controlling them if we can better predict how they spread and respond to different interventions. Toward this goal, we developed a modeling framework using fine-grained satellite data on areas of human settlements and high-resolution population data to build a road-connected network upon which to simulate disease spread (Figure 6). Prior to modeling disease spread, we incorporated mobility patterns in this network by estimating the commuting rates between the road-connected settlement/urban areas. We demonstrated this approach for Rwanda and simulated the spread of the 2009 pandemic H1N1 influenza across the constructed network.

The spread of pandemic influenza A H1N1 in Rwanda was simulated via an SEIR (susceptible, exposed, infectious, recovered) compartmental model, where the disease spread was simulated both within and between urban areas. In the SEIR model, a susceptible person becomes infected upon contact with an infectious individual and transitions to the exposed or latent compartment, after which the individual becomes infectious (symptomatic or asymptomatic) and subsequently recovers with immunity to future infections. The disease transmission parameters used in the model were obtained from existing literature on pandemic influenza.1,2,3,4

The simulated outbreaks closely followed the actual influenza outbreak in terms of forecasted outbreak length and the order in which areas were infected. The modeling framework developed above has been used to write an R5 package which facilitates the application of this model to other diseases. In real outbreak scenarios, this tool can be valuable for risk-based disease surveillance, emergency planning, and control purposes. With its help, policy makers and outbreak responders can determine the spread of an outbreak to best decide areas for targeted control. In addition, by simulating the effects of control measures, such as vaccination, public health officials can decide on the most beneficial intervention scenarios with respect to both the probability of an outbreak occurring, as well as its spread and impact. Finally, by using satellite imagery and available GIS data, it is possible to apply this model to different and possibly even remote regions of the world, including areas with higher risks of emerging infectious diseases.

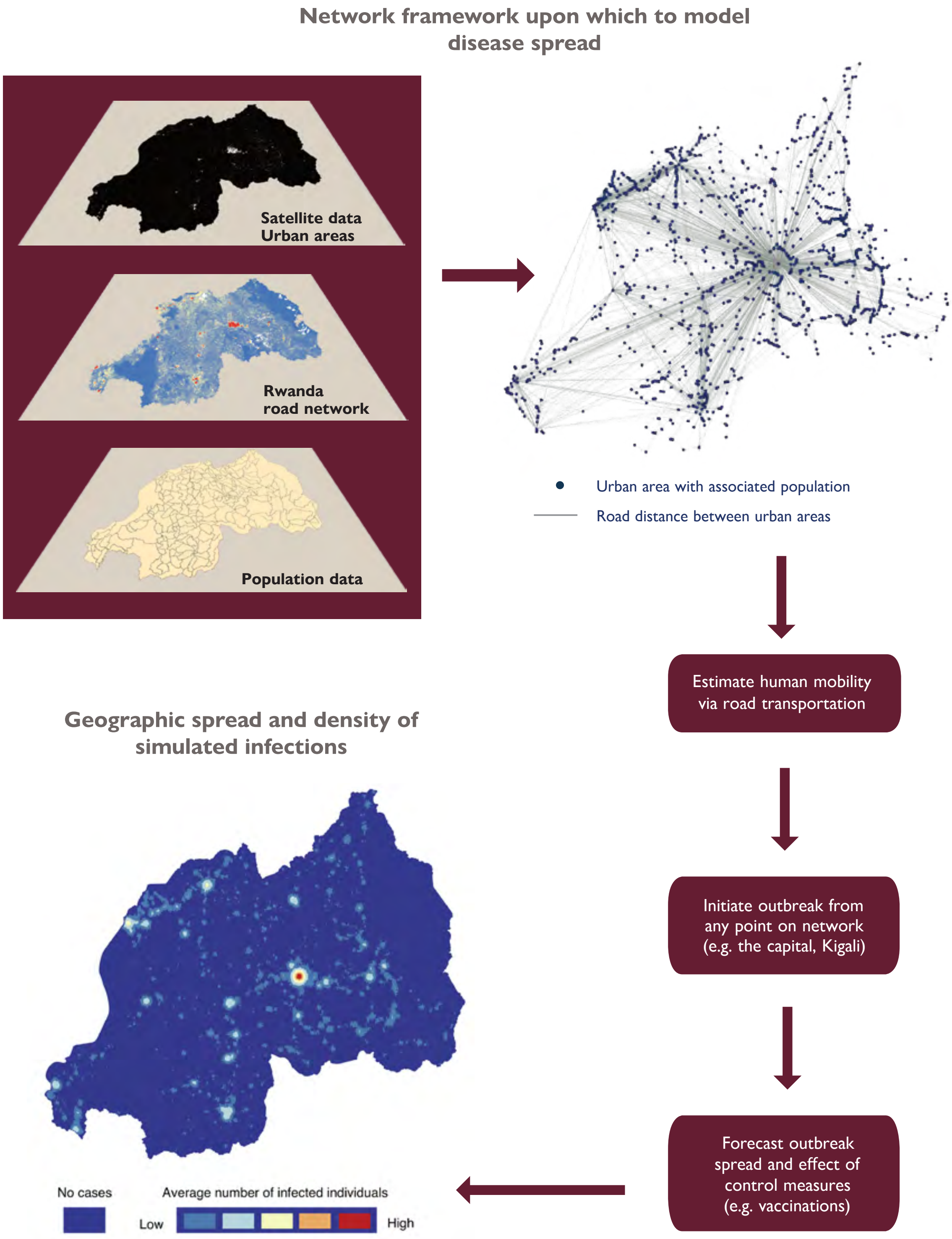


FIGURE 6. ***Network creation, disease simulation process, and map of influenza cases over the course of simulated outbreaks in Rwanda.***

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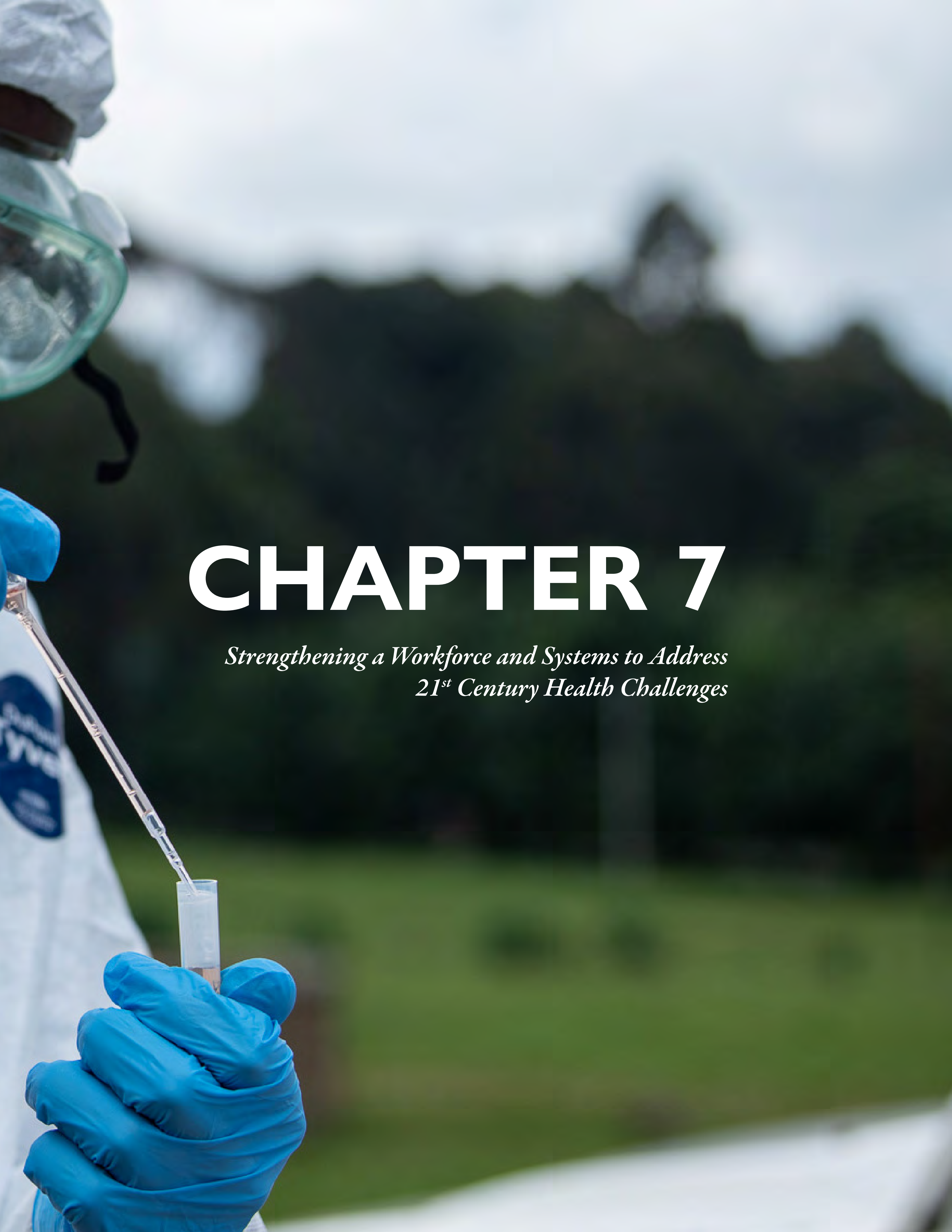
PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS



CHAPTER 7

Strengthening a Workforce and Systems to Address 21st Century Health Challenges

KEY TAKEAWAYS

- The PREDICT project trained more than 6,000 One Health professionals around the world.
- We transformed how laboratories and health systems approach zoonotic disease surveillance.
- Our teams improved critical infrastructure necessary for field surveillance and laboratory testing of emerging and zoonotic disease threats.
- PREDICT contributed to development and implementation of One Health platforms, policies, and structures.
- One of the most durable legacies of PREDICT will be the people and the systems the project worked to support and strengthen.

PREPAREDNESS

Networked with collaborating laboratories globally to share data, protocols, and foster international colllaborations. Additionally, connected scientists in PREDICT-participating countries and provided training, testing protocols, and funding for supplies and personal protective equipment.

EARLY WARNING

Provided the serological evidence that people living at the wildlife-human interface in rural China are being exposed to SARS-related coronaviruses - marking them as a clear and present danger, suggesting that intermittent spillover could be occurring.

PREDICT-trained personnel were on the front lines of COVID response and research in many countries

EARLY DETECTION

Helped raise the flag that coronaviruses have pandemic potential by providing critical data on the group of coronaviruses to which SARS-CoV-2 belongs, through collaborations with the country teams and with National Institutes of Health (NIH).

EARLY DETECTION

Utilized available and cost-effective consensus-based PCR protocols to broadly detect viruses for early detection of SARS-CoV-2 in several countries. Our network supported one another in interpretation of results and optimization of the assays for early detection of the first COVID-19 cases before a specific assay targeting the novel coronavirus was available.

In late December 2019, health authorities began treating dozens of cases of mysterious pneumonia-like illness in Wuhan, China. In early January 2020, Chinese researchers identified the cause of the illness as a new coronavirus and shared the genetic sequence with the global community. The prior findings of coronaviruses in the region, to which PREDICT contributed significantly, helped to put the virus into context and provide critical information to assess zoonotic risk. As news of the virus began to spread, so did people from Wuhan, traveling throughout China and beyond.

It was inevitable, this is what we trained for.

In Thailand, health authorities began screening travelers arriving from China and admitting patients with high fevers into hospitals. Samples from these travelers arrived at the Chulalongkorn University lab for testing, a PREDICT partner lab with a decade of experience detecting known and emerging viruses. Within days, they detected the new coronavirus using PREDICT's protocols, the first time this virus was recorded outside of China.

In another breakthrough, the team used the short-fragment of viral RNA to design a more specific and rapid genetic test, anticipating the potential surge in cases and demand for testing at scale. In nearby Cambodia, the Institut Pasteur team, another partner connected to Chulalongkorn through PREDICT's One Health network, consulted with Thai colleagues to modify coronavirus detection protocols for the new virus. Across the Himalayas in Kathmandu, Nepal, the Center for Molecular Dynamics was also called into action to test samples from suspect patients, and with support from the UC Davis lab, they confirmed that the new virus had reached South Asia.

*We are part of the workforce on the frontlines
of pandemic response and prevention.*

One of PREDICT's greatest legacies is the people and the systems our project worked to support and strengthen. Through collaboration with communities and governments at the local, national, and regional levels, and dedicated training for the current and future workforce in One Health skills to prevent and respond to pandemic threats, the countries where PREDICT worked are better prepared to address health threats of the 21st century. Active engagement across policy domains helped reinforce national and multi-sectoral health security infrastructure across PREDICT countries, in many cases building new partnerships and informing pathways to advance a "health security in all policies" approach. The value of PREDICT's investment in capacity building and systems strengthening is no better exemplified than in the response led by PREDICT teams to the COVID-19 pandemic around the world.

Through USAID investments, doctors, veterinarians, lab technicians, ecologists, and social scientists trained through PREDICT are recognized as experts in preventing, detecting, and responding to emerging viral threats. The Chulalongkorn lab in Thailand was one of more than 50+ labs conducting COVID testing in-country. The Institut Pasteur lab in Cambodia tested cases at government request, including passengers that were quarantined on the Westerdam cruise ship docked offshore. In Tanzania, the Ifakara Health Institute team was called into action as a critical testing hub, serving as a training leader for ministry partners, and establishing SARS-CoV-2 testing capacity for laboratories on Zanzibar and as far away as Equatorial Guinea. Using a similar approach in Ghana, the Noguchi Memorial Institute for Medical Research quickly ramped up testing in March as the primary national testing center. Also critical to the test and trace strategy for COVID-19 control in Sierra Leone, PREDICT's team has been active at the national level, leading contact tracing efforts in multiple districts and communities. These are the professionals transforming their country's health systems. This is the workforce we depend on for global health security.

BUILDING A ONE HEALTH WORKFORCE

> “Surveillance gaps at the individual country level can affect global health.”
>
> —Schwind et al., 2014

The PREDICT project focused its efforts in regions of the world considered “hot spots,” countries where emerging and re-emerging zoonotic diseases are most likely to appear due to high human population density and high biodiversity, among other factors. In low- and middle-income countries, there are additional vulnerabilities in health systems for surveillance and detection of these disease threats, including inadequate human resources for disease detection and control, and so increasing human capacity was a priority for our targeted engagement.

Implementing a One Health approach to disease surveillance requires a well-trained, technically skilled, and multidisciplinary workforce. Surveillance teams need the knowledge and capabilities to safely and humanely collect samples from animals and people, conduct the laboratory tests that identify a virus and its corresponding health risks, and explore the social and behavioral factors associated with viral spillover and spread.

PREDICT invested in the infrastructure needed to develop this workforce, with a tailored curriculum and training program that included protocols covering 19 One Health-related topics critical for project implementation and long-term sustainability in the Africa and Southeast Asia regions for over a decade, and in Latin America for the first few years.

THE FIRST FIVE YEARS

Since 2009, PREDICT has established and sustained One Health disease surveillance trainings using online, workshop style, and hands-on experiential formats. In the first five years of the project, PREDICT-1 provided training for most aspects of wildlife disease surveillance, as safe wildlife handling and disease surveillance skills, as well as laboratory competencies to diagnose known and novel viruses, were very limited in most countries. PREDICT-1 trained over 2,500 students, health care providers, veterinarians, natural resource managers, laboratory technicians, and government personnel on topics including biosafety, safe wildlife disease surveillance, laboratory techniques, and disease outbreak investigation. PREDICT-1 teams also worked with communities and local stakeholders to raise awareness around zoonotic disease risks and risk reduction measures.

As part of evaluating wildlife disease surveillance effort priorities and programmatic needs, PREDICT-1 conducted informal structured assessments with local experts and stakeholders to identify key gaps in national health systems, including human, laboratory, institutional, and coordinating capacities that, if addressed, would help improve surveillance systems for emerging viral threats from wildlife. Critical interfaces indentified to focus future efforts on included points along bushmeat value chains (particularly hunting and butchering) and areas of land use change. PREDICT-1 scientists and stakeholders also identified key challenges in wildlife disease surveillance, as outlined in Figure 1. These findings helped the PREDICT team refine the approach and to focus further on health systems capacity building in PREDICT-2. This assessment also confirmed the importance of improved coordination, collaboration, and communication among stakeholders, as well as human and laboratory capacity building.

In conclusion, the authors of the structured assessment recommended the following: *“a One Health approach to capacity building that improves zoonotic pathogen surveillance in wildlife at local and global scales is greatly needed. This approach will include building bridges across ministries and sectors to enable sufficient manpower and funding mobilization to facilitate efficient targeting of high risk interfaces for zoonotic disease transmission.”* This set the stage for PREDICT-2 focused capacity building efforts in the pursuit of One Health surveillance of humans, wildlife, and domestic animals at high risk interfaces.

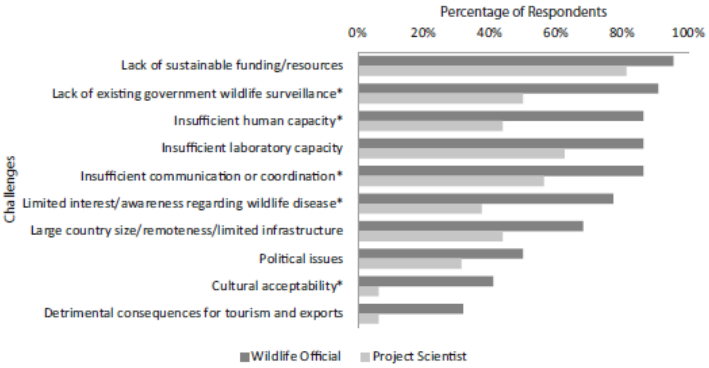


FIGURE 1. ***Challenges associated with conducting zoonotic pathogen surveillance ranked by wildlife officials and project scientists as "important."***

**Indicates a statistically significant difference between perspectives among two stakeholder groups. (Schwind et al., 2014)*

OUR APPROACH (2014-2020)

PREDICT-2 operationalized One Health surveillance in 2014-2020 through strategic concurrent surveillance of humans, wildlife, and domestic animals at high-risk interfaces across Africa and Asia. This approach brought together domains that spanned national health systems and extended across other ministries and relevant stakeholders. These collaborative efforts required targeted investment in human, laboratory, and institutional capacity building to ensure that the research and community engagement efforts were safe, ethical, and aligned with project objectives. Furthermore, PREDICT teams engaged across all levels of society, contributing to subnational, national, and international One Health platforms and policy discussions. In many countries, PREDICT contributed to policy development and governance structures that will help ensure the One Health approach remains embedded within national global health security initiatives.

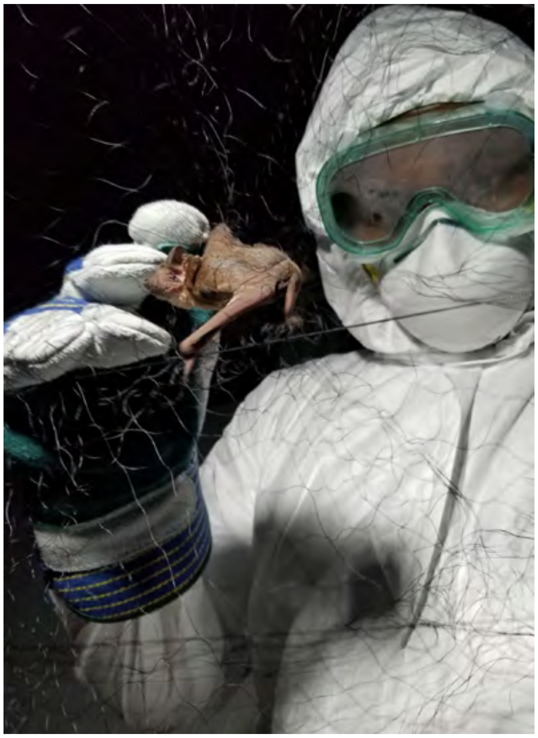


PHOTO: PREDICT TANZANIA

STRENGTHENING CAPACITY

TOTAL TRAINED

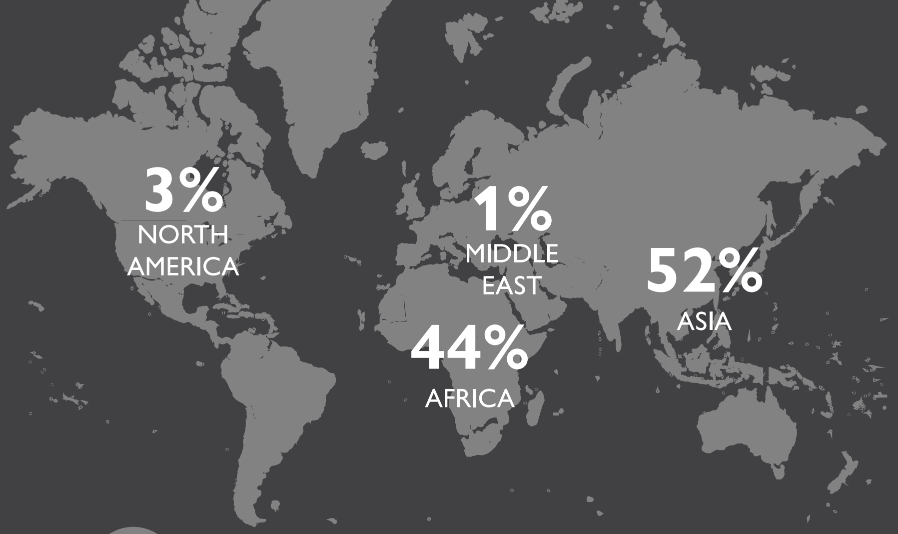
3,857*

INDIVIDUALS

(**716** STUDENTS)

41% WOMEN

59% MEN



25% GOVERNMENT / MINISTRY

7% RESEARCH INSTITUTE

24% UNIVERSITY

11% OTHER

20% NGO

13% GOVERNMENT LAB

20,341

INDIVIDUAL INITIAL & RENEWAL TRAINING MODULES COMPLETED

42%	SAFE ANIMAL SURVEILLANCE
28%	SAFE LABORATORY PRACTICES FOR VIRUS DETECTION
12%	HUMAN SURVEILLANCE & BEHAVIORAL RISK ASSESSMENT
11%	EMERGENCY PREPAREDNESS & OUTBREAK RESPONSE
6%	ONE HEALTH APPROACH, POLICIES, PLANS
1%	INFORMATION MANAGEMENT, DATA INTERPRETATION, GEOSPATIAL ANALYTICS, OTHER

* = Number trained reflects PREDICT-2 training numbers; an additional 2,500 individuals were trained under PREDICT-1

A ONE HEALTH LEARNING LIBRARY FOR STUDENTS & PROFESSIONALS

To encourage sharing of the knowledge and skills essential for safe and effective One Health surveillance, detection, and characterization of zoonotic disease threats, PREDICT's training materials, protocols, and e-book resources are freely available to the public (in English and French) at: **www.publications.predict.global**

PUBLICLY AVAILABLE PREDICT GUIDES & PROTOCOLS:

BIOSAFETY, COLD CHAIN, AND EMERGENCY PREPAREDNESS RESOURCES

- Basic Laboratory Safety
- Biosafety & PPE Use
- Emergency Preparedness
- Implementing Cold Chain for Safe Sample Transport & Storage
- Packing & Shipping Biological Samples

ONE HEALTH SURVEILLANCE & FIELD SAMPLING GUIDES

- Avian Sampling Methods
- Bat Sampling Methods
- Bushmeat Sampling Methods
- Livestock Sampling Methods
- Non-Human Primate Sampling Methods
- Rodent Sampling Methods
- Safe Animal Capture & Sampling
- Small Carnivore Sampling Methods

BEHAVIORAL RISK & QUALITATIVE RESEARCH GUIDES

- Qualitative Research: Introduction & Observational Research Methods
- Qualitative Research: Focus Groups, Ethnographic Interviews & Data Analysis

STANDARD OPERATING PROCEDURES FOR ONE HEALTH SURVEILLANCE*

- General Information
- Field Sampling Guides
- Qualitative Research Guides
- Training Quizzes

PREDICT's Standard Operating Procedures for One Health surveillance are available for digital download at* **p2.predict.global/predict-protocols

HEALTH SYSTEM STRENGTHENING & OPERATIONALIZING ONE HEALTH

PHOTO: CRISTIN WEEKLEY

As the largest operational global One Health project to date in terms of topical and geographic scope, PREDICT worked with partners to shift from largely conceptual interest in multisectoral collaboration to implementation and institutionalization of One Health. These ground-truthed One Health approaches and lessons learned provide a critical evidence base that can be adapted and upscaled to other contexts.

As evidenced by the global impact and reaction to the COVID-19 pandemic, adequate prevention, detection, and response to emerging health threats of the 21st century require evolving our health paradigm to systems that are more nimble, comprehensive, and coordinated and that expand beyond the traditional domains of a health system. Grounded in a One Health approach, PREDICT strengthened capacity across many domains and levels commonly prioritized in health systems, and also expanded implementation models and scope for One Health approaches. PREDICT helped build evidence for the value that putting One Health in action can bring to addressing complex problems.

PREDICT focused on multidisciplinary experiential training opportunities for veterinary, human health, wildlife, and environmental professionals. Uniting experts across sectors to foster collaboration and communication, to help break down disciplinary silos and to enhance problem solving skills, dramatically increased capabilities for readiness and response to newly emerging diseases and outbreaks of unknown origin. PREDICT strengthened the human resources in each country and region through training numerous scientists, professionals, and ministry personnel. This capability strengthening was implemented primarily through in-service training, which took advantage of ongoing project activities associated with disease surveillance in the field, laboratory testing to identify known and new pathogens, data and epidemiologic analyses, outbreak response, and community engagement. In-service training was often complemented by workshops and tabletop exercises for government workers, community stakeholders, faculty, and students.

PREDICT's capacity building efforts spanned domains and silos, and impacted many facets of existing health systems, including individuals, organizations, laboratory networks at subnational, national, and regional levels, governance structures, regulatory bodies, and policies. After a decade of collaborative activities, new leaders have emerged from the project, as beneficiaries of years of intensive training, networking, and on-the-job experience. These individuals are already taking the helm and integrating the One Health approach into global health security initiatives in their institutions, countries, and regions.

ONE HEALTH CHAMPIONS AROUND THE WORLD

JAMES BANGURA

PREDICT Sierra Leone,
Country Coordinator

Prior to joining the PREDICT project, James worked as a lead surveillance officer for the government of Sierra Leone battling on the front lines of the West Africa Ebola epidemic. For James, this devastating outbreak highlighted the importance of engaging communities, increasing awareness of zoonoses, advocating for civilians to take control of their personal health, and providing guidance on ways to reduce the risk of zoonotic disease transmission.

While leading PREDICT efforts in Sierra Leone, James worked to strengthen capacity by providing technical support on disease surveillance to the Ministries of Health and Agriculture at the national level, mobilizing district level outbreak preparedness and response efforts, and engaging with local communities around the country. James is now part of a team of professionals providing critically needed technical assistance to the government of Sierra Leone during the COVID-19 outbreak. He and his colleagues are leading contact tracing efforts, informing people of their exposure to COVID-19, and advising them on how to safely isolate, seek care, and obtain social services. By directly engaging the community in response efforts, James is continuing to ensure that communities are empowered to make informed decisions on how to reduce their risk.

> “Bringing what we know to the community brings [me] fulfillment – I tell the community ‘this is what we have seen and most likely what we will find, what we are looking for, and what you are doing that could expose you.’ Sometimes they had no idea, no one told them, and they are thankful [for our guidance].”
>
> —James Bangura

CAPTAIN FORTUNE EBEN YOUMBA

PREDICT Cameroon,
Behavioral Risk Investigator

Captain Fortune Eben Youmba joined the PREDICT Cameroon team as an intern after she graduated with her Doctor of Veterinary Medicine degree in 2015. During her internship, Captain Eben benefited from on-the-job training, including biosafety and safe animal sampling in the field and virus detection at the Military Health Research Center (CRESAR). She also took part in behavioral risk training and was selected as a member of the behavioral risk investigation team for PREDICT Cameroon. Captain Eben demonstrated strong social science skills and an aptitude for conducting interviews to gain insight into risk factors for disease emergence among communities. Following her internship and officer training, she was assigned to CRESAR by presidential decree where she worked with PREDICT and continued to strengthen the capacity of the Ministry of Defense in outbreak investigation and response. Captain Eben brings critically needed expertise and skills to the workforce at CRESAR, an important node in the national system for zoonotic disease prevention, detection, and response and one of the key national laboratories for emerging infectious disease detection.

SUPAPORN WACHARAPLUESADEE

PREDICT Thailand, Country Coordinator & Laboratory Chief

For the past two decades, Supaporn Wacharapluesadee has been working in settings where bats and humans come into close contact with each other to improve our understanding of the distribution, reservoirs, and spillover risk of some of the most deadly zoonotic viruses in people. Supaporn joined the PREDICT team in 2009 and led surveillance and virus detection efforts in Thailand. Through her role as the Thailand Laboratory Chief for PREDICT, she rapidly built the capacity in her laboratory at Chulalongkorn University to detect both known and unknown viruses in animals and people.

Currently, in addition to her work as a faculty member at Chulalongkorn University, Supaporn is the Lab Chief for the WHO Collaborating Centre for Research and Training on Viral Zoonoses, and her lab serves as the national reference laboratory for rabies virus, MERS-CoV, Ebola, Zika, and other infectious disease diagnoses for the Ministry of Public Health. Supaporn was the first scientist to detect the novel SARS-CoV-2 outside of China and rapidly alerted the government of its arrival to Thailand. Her expertise in applying molecular platforms to discover and characterize novel pathogens was critical for early detection and response to this emerging pathogen.

> “The Thailand team and I have learned and gained valuable experiences from our work with PREDICT, practiced until the unknown was known, and built relationships and trust among partners.”
>
> —Supaporn Wacharapluesadee

PHAM THI BICH NGOC

PREDICT Viet Nam, Veterinary Program Officer

An emerging One Health leader in Vietnam, Ms. Pham Thi Bich Ngoc is a 2018 graduate from the Faculty of Veterinary Medicine, Vietnam National University of Agriculture. As a veterinary program officer with PREDICT/Viet Nam, she's had hands-on opportunities to gain knowledge and experience with the One Health approach to zoonotic disease surveillance. Her expertise is invaluable, as different sectors within Vietnam collaborate on the development of national standard operating procedures for wildlife disease surveillance and event reporting.

Ngoc is also an alumni of the 2019 Rx One Health Field Institute, a month-long experiential learning course held in Tanzania focused on developing critical One Health core competencies. With a solid foundation in field and laboratory techniques and effective problem solving skills, Ngoc is well positioned to become a strong female leader and advocate for the One Health approach in Vietnam.

> “Being a junior staff in PREDICT Viet Nam has helped strengthen my knowledge of the dynamics of zoonotic virus evolution, spillover from animals to human, amplification, and spread; my skills in identifying the “hot spots” of high-risk disease transmission interfaces to address wildlife trafficking in the region; and my ability to help prevent the devastating and destabilizing effects of a disease pandemic.”
>
> —Pham Thi Bich Ngoc

MEET MORE ONE HEALTH LEADERS / FACES OF PREDICT **p2.predict.global/faces-of-predict**

BUILDING CAPACITY FOR RISK ANALYSIS

An understanding of patterns of risk factors and disease transmission informs risk management and disease control strategies. The ability to describe, map, and model data is therefore imperative for One Health surveillance activities. Together with our global disease modeling and analytics teams, we provided training on basic data analysis tools, spatial mapping, and disease modeling to PREDICT project partners through fellowship programs, workshops, and an innovative, collaborative data conference in the US. These trainings strengthened the skills of young and practicing scientists around the world, empowering them with the knowledge and skills to assess and analyze risk and to use data to inform disease surveillance and control policies and interventions.

MODELING & ANALYTICS FELLOWSHIPS

NEW YORK, USA

Two extended fellowships were completed with members of the PREDICT Ghana and Thailand teams. This fellowship, hosted by Consortium partner EcoHealth Alliance, focused on analysis of project data to inform strategic policy and intervention recommendations. In addition, two members of the PREDICT Indonesia team were hosted by EcoHealth Alliance for an accelerated fellowship program focused on the analysis of project wildlife and behavioral risk data.

RISK CHARACTERIZATION WORKSHOP

BRUSSELS, BELGIUM - JANUARY 2018

This extensive workshop, held for all members of the PREDICT Consortium during our annual meeting, focused on analysis of emerging infectious disease risk maps, integrating data from One Health surveillance activities, and exploring emerging insights from data collected through behavioral risk investigations. The goal was developing actionable surveillance improvements and risk mitigation strategies, and outcomes from the workshop informed each country's One Health surveillance plans.

PHOTOS: EUNAH CHO PRESTON

DATA CONFERENCE FOR PREDICT COUNTRY TEAMS

USA - JULY 2019

The goal of the PREDICT-2 Data Conference was to introduce PREDICT-2 staff to skills to better understand, visualize, analyze, and interpret their own country's PREDICT-2 data. Held in June 2019, this conference brought together over 40 global and country PREDICT team members from 12 PREDICT countries, including Cambodia, Ghana, Guinea, Nepal, Rwanda, Senegal, Sierra Leone, Tanzania, Uganda, Kenya, Myanmar, and Vietnam. Through a week-long interactive mix of lectures and small group activities, participants gained skills in data analysis, GIS mapping techniques, hypothesis testing, manuscript preparation, and risk assessment.

2019 PREDICT Data Conference organizers and participants

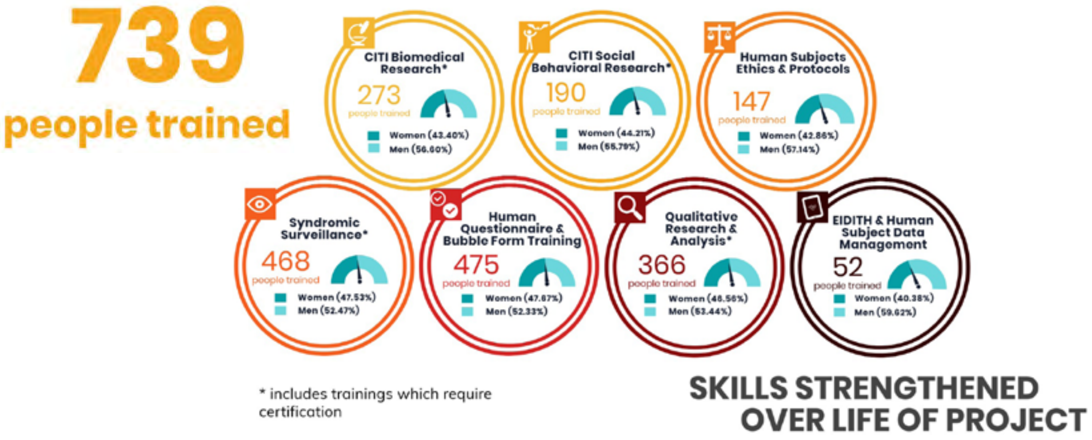
PHOTO: DON PREISLER, UC DAVIS

> "What I liked best were the practical applications to our current work, especially data analysis and preparation of presentations."
>
> —Conference participant

> "I found the tutorials on R and QGIS to be very useful. I was intimidated about using it, as I never had previous exposure, but the step by step instructions and guidance from the conference organizers made both programs easier to use."
>
> —Conference participant

BUILDING BEHAVIOR RISK ASSESSMENT CAPACITY

From 2014-2019, we trained 732 local scientists and professionals across 28 countries in behavioral science methodologies and ethical human subjects research and launched standardized behavioral risk investigations that were adapted to each countries' specific contexts and high-risk interfaces.



INSTITUTIONAL & ORGANIZATIONAL LEVEL CAPACITY STRENGTHENING

Recognizing the need for a more proactive approach to disease prevention and control by discovering and mitigating pathogens at their source before they spillover into people, PREDICT designed a surveillance strategy targeting high-risk disease transmission interfaces where people come into close and frequent contact with wildlife. Over the first five years (2009-2014), PREDICT teams sampled wildlife considered most likely to serve as reservoirs for emerging zoonotic pathogens at these high-risk interfaces. In 2014, this approach was ramped up using a standardized One Health surveillance and virus detection strategy for collecting and screening concurrent samples from animals, as well as from people at the same study sites, with the goal of identifying viruses with zoonotic potential, factors that increase the risk of spillover, causes of undiagnosed acute febrile illness in humans, and strategies to decrease risk of cross-species transmission and spread.

Over the life of the project, PREDICT strengthened capacities in many institutions and organizations that play a key role in national and global health security. For instance, PREDICT improved diagnostic capabilities in government, university, and private laboratories that are critical for strengthening national laboratory systems. These investments are already improving the abilities of countries to rapidly detect and respond to biological threats.

SUBNATIONAL & NATIONAL LEVEL

In East Africa, PREDICT had a high level impact by supporting teams at two well-positioned laboratories in Ethiopia that were linked to the national laboratory system representing the animal and the human health sectors, and new technology as well as human capacity was established to conduct field surveillance and molecular diagnostics for viral family screening. The in-country personnel were trained by global and regional experts in topics such as biosafety, cold chain techniques, and virus detection protocols. These investments were valuable to equip and empower ongoing surveillance for both known and emerging viral threats in the country.

Similarly in Jordan, the PREDICT team played a critical role in the development of the molecular and virology laboratory at the Jordan University of Science and Technology (JUST). PREDICT Jordan team's success was evidenced by the JUST laboratory's ability in 2018 to detect an emerging virus, koi herpes virus, which had caused a large die-off of fish in the country. Training students using on-the-job internship placements and short term hires has built their skills and their competitiveness in the job market.

PREDICT has also strengthened surveillance and virus detection capabilities in South Asia such as in Nepal, where the Center for Molecular Dynamics Nepal (CMDN), established in 2007, is now recognized as a center of excellence in public health and wildlife disease research in the country. When COVID-19 emerged in China, CMDN joined the government in its surveillance and response efforts, assisting with testing suspect cases and aiding in early detection in Nepal. CMDN is now building on these efforts with the application of a method to detect SARS-CoV-2 in environmental samples (e.g. sewage) as an innovative means to assist with monitoring community transmission across the country.

PREDICT disease surveillance strategies were designed and updated in partnership with in-country government partners and stakeholders. Working hand-in-hand with the Ministries of Health, Agriculture, and Environment in each of the countries to identify and prioritize the sites and species to target for our surveillance activities, PREDICT partnered with local communities and health offices at the subnational and national levels to engage with patients in health care facilities, as well as people in villages considered high-risk for zoonotic disease emergence.

In these communities, multi-sectoral teams representing human, animal, and environmental health worked together to safely to conduct concurrent surveillance for humans, wildlife, and where feasible, livestock and domestic animals. PREDICT findings have subsequently informed policies and intervention recommendations that strengthen health security. For example, in Cambodia PREDICT engaged government partners from the Cambodia CDC, Ministry of Agriculture, and the Forestry Administration in a coordinated One Health approach in which a multi-sectoral team, comprised of staff from these organizations, conducted the first ever concurrent sampling of wildlife, domestic animals, and people in Cambodia to investigate risk associated with cross-border rodent trade and bat guano farming.

In Thailand, PREDICT partnered with FAO and the Department of Livestock Development to conduct triangulated human, wildlife, and domestic animal surveillance in areas where Nipah virus has been previously found, and behavioral risk is being investigated in high-risk communities to complement the laboratory risk data. Similarly, in Ghana, a multidisciplinary team of staff from the Wildlife Division of the Forestry Commission, Veterinary Services Directorate, Ghana Health Service, and Noguchi Memorial Institute of Medical Research worked together to sample wildlife and people in agricultural communities where people come into close contact with fruit bats and rodents in their orchards and fields. Samples were tested at in-country government and university laboratories using a standardized approach in which the human and animal samples were tested for the same viral families using the PREDICT testing protocols.

To complement PREDICT's surveillance activities and build on early findings, community outreach campaigns were conducted, often led by health promotion officers and wildlife officials, to increase awareness and promote risk reduction strategies at the community level. The added value of these multi-sectoral partnerships and the subsequent buy-in from stakeholders has led to the institutionalization of One Health approaches in some settings. In Cambodia and Ghana for example, this success is evidenced by the ongoing application of a One Health surveillance approach in disease outbreak investigations and the uptake of PREDICT's virus detection protocols in laboratories used by the government to investigate undiagnosed causes of illness in humans and animals.

Throughout the life of the project, PREDICT was responsive to specific needs and requests by the governments to address gaps in workforce capacities. For instance, PREDICT Cameroon worked in partnership with FAO to address a gap in national wildlife disease surveillance capacity identified through the WHO Joint External Evaluation (JEE), an assessment conducted by Cameroon to assess the country's health security strengths and weaknesses. The team conducted intensive training on wildlife disease surveillance for staff from the Ministries of Wildlife and Livestock. In addition, the One Health Focal Point from the Ministry of Forests and Wildlife joined the PREDICT team to train game rangers from protected areas around the country. PREDICT also participated in curriculum development for a Master of Science degree in Wildlife Health at the University of Buea as a sustainable mechanism for enhancing capacity in the One Health workforce in Cameroon through building a pipeline of professionals with wildlife expertise.

OUTBREAK INVESTIGATION TECHNICAL ASSISTANCE

PREDICT also provided critical technical assistance during disease outbreak investigations when requested by the national governments. Our teams worked with key stakeholders to operationalize the One Health approach during the response. For example, the PREDICT team assisted the Bangladesh government with investigation of a disease outbreak in crows, which provided opportunities for PREDICT to train a multidisciplinary government team, including officials from the Department of Livestock Services (DLS), on biosafety and avian sampling methods. The Government of Bangladesh outbreak team led the response, and the crow samples tested positive for H5N1 avian influenza at the Bangladesh Livestock Research Institute (BLRI) laboratory. The team's rapid mobilization demonstrated improved outbreak response capacity, which especially for wildlife diseases, is a critical gap in Bangladesh's national surveillance and detection systems.

PREDICT also assisted the Bangladesh government with investigations of encephalitis outbreaks that were suspected to be caused by Nipah virus. For these outbreaks, PREDICT joined the investigation team with the Institute of Epidemiology, Disease Control & Research (IEDCR) that was leading the investigation of the human cases. The PREDICT team worked synergistically by collecting samples from *Pteropus medias* fruit bats (known Nipah virus reservoir) roosting near the residences of the suspect cases.

Similarly in Africa, PREDICT participated in a joint investigation of Lassa fever in Ghana. The team conducted field investigations at sites where the suspect case had resided prior to his death. In collaboration with the Ghana Health Service and FAO, PREDICT personnel from the Wildlife Division of the Forestry Commission and Veterinary Services Directorate conducted the field investigations, safely sampling rodents and administering questionnaires to evaluate human practices that could put this community at greater risk of exposure. The Noguchi Memorial Institute of Medical Research screened the rodent samples for arenaviruses and Lassa fever virus, and PREDICT worked with the Ghana Health Service and the School of Public Health to educate the community on zoonoses and strategies to reduce their risk. The government of Ghana viewed this effort as a One Health success story in which personnel representing the three ministries worked collaboratively to investigate the outbreak and conduct community education and outreach.

PREDICT country teams' continued roles in conducting surveillance and testing in support of the governments' responses to disease outbreaks, including the current COVID-19 outbreak, highlight how this trained workforce is prepared to assist with the response to known as well as emerging viral threats. In Tanzania, a cadre of PREDICT's women scientists have led training efforts to help increase SARS-CoV-2 testing capacity in Tanzania and beyond.

Read more about PREDICT"s role in Outbreak Response and Investigation in *Chapter 6*.


PHOTO: PREDICT BANGLADESH


PHOTO: PREDICT TANZANIA

REGIONAL COORDINATION & COMMUNICATION

Beyond creating a global network of One Health professionals, PREDICT conducted strategic regional south-south training exchanges to share knowledge and develop communities of practice. For example, PREDICT promoted collaboration between Egypt's National Research Centre's Center of Scientific Excellence for Influenza Viruses and the Jordan University of Science and Technology through joint training and sample/data sharing. The PREDICT Egypt team traveled to Jordan for hands-on training in safe bat capture and sampling. In turn, the PREDICT Jordan team assisted with safe and effective implementation of One Health surveillance in at-risk Jordanian communities. In addition, PREDICT lab teams worked together to better understand exposure to Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Human samples from both countries were screened for MERS-CoV-neutralizing antibodies, and data were shared across institutions. This collaboration facilitated regional insights on MERS exposure in the North Africa and Middle East regions.

In addition, PREDICT teams in East Africa who received training in the early years of PREDICT-1 stepped up in 2014 as regional trainers to work with new team members, such as those from West Africa who joined during the second phase of PREDICT. For example, the PREDICT Senegal and Ghana teams received training and technical assistance from their colleagues in Rwanda, Tanzania, and Uganda in order to strengthen their capacity to conduct zoonotic disease surveillance. During these training sessions, experienced scientists and veterinarians from Rwanda, Uganda, and Tanzania provided new surveillance teams with hands-on instruction on how to safely and ethically implement PREDICT's surveillance activities in the field and in partnering health care facilities. These types of regional capacity exchanges helped establish lasting professional networks that fostered collaboration and communication that transcended borders and disciplines.

When COVID-19 first emerged, PREDICT team members who had been linked over time as a global network of professionals, rapidly started sharing information and resources through social media platforms, launching impromptu virtual communities of practice. These communities have provided an efficient mechanism to share best practices and lessons learned while working on the frontlines of this pandemic. Early communications between PREDICT scientists in laboratories in Thailand and Cambodia helped both of these countries troubleshoot testing protocols and accelerated the pace of both countries being able to successfully test for SARS-CoV-2, helping their countries at a critical time in the earliest stages of the pandemic. Our established networks supported one another in interpretation of results and optimization of the assays for early detection of the first COVID-19 cases before a specific assay targeting the novel coronavirus was available.

A REGIONAL APPROACH TO OVERCOMING CHALLENGES

In many PREDICT countries, there were similar challenges to overcome in order to cultivate a One Health workforce ready to collaborate across disciplines and sectors, especially when basic things like power, cold chain supplies, and inconsistent administrative requirements presented regular obstacles. As solutions for these basic challenges became apparent in one country, solution ideas quickly spread to other country teams, and so the PREDICT network solved problems together more quickly than they would have separately. Whether the solutions related to improving backup power supplies and access to liquid nitrogen or to finding ways to work effectively offline in remote areas and finding ways to work across timezones online to troubleshoot problems, the PREDICT teams repeatedly rose to the occasion to respect, trust, and collaborate over time. Beyond overcoming seemingly basic challenges, there were of course complex challenges related to policies, politics, and people that must always be handled delicately to find the best path forward. There is often not a "one size fits all" solution, and hence the importance of open communication and rapport across partners became rapidly apparent for finding optimal solutions that resulted in a more competent and collaborative workforce, ready to use a One Health approach to address global health security.

EMPOWERING WOMEN IN SCIENCE AND STRENGTHENING COVID-19 DETECTION IN EAST AND WEST AFRICA

CONTROLLING A DEADLY DISEASE BEGINS WITH DETECTION

For millions of Tanzanians, detecting a disease threat such as SARS-CoV-2, the virus that caused the current COVID-19 pandemic is in the hands of brilliant female scientists supporting the national laboratory system, scientists such as Ms. Sarah Mswata, the laboratory Manager, Dr. Grace Mwangoka, Zena Emmanuel, Ms. Happy Mkali and Ms. Tunu Mwamlima. These women are among the lead lab scientists at the Ifakara Health Institute's (IHI) Bagamoyo Research and Training Center, next door to the District Hospital in Bagamoyo town.

For the past 6 years, Sarah, Grace, Happy, Tunu, and Zena were the core team leading the USAID PREDICT project's human surveillance activities in Tanzania, designed to investigate and detect emerging disease threats from wildlife, zoonotic diseases such as Ebola, Influenza, SARS or MERS. Working closely with health centre staff and directly with local communities in the Kigoma and Kagera regions, along with PREDICT partners at the Sokoine University of Agriculture (SUA), they put the One Health approach in action to explore the multiple dimensions of emerging disease risk. They also trained district-level veterinary and public health professionals in the safe collection of samples from wild animals and people that live among them, and they tested these samples for viral threats in their research labs.

Between 2009-2019, the IHI and SUA labs conducted >34,000 tests and detected 77 unique viruses including 60 new viruses

Recognized as centers of excellence for disease detection, the IHI and SUA labs were among those selected and validated to support national testing for SARS-CoV-2. The IHI lab in particular has risen to the occasion, independently providing technical assistance and expertise to expand COVID-19 detection beyond the mainland to the public health labs on Zanzibar. In July 2020, five lab technicians (two men and three women) from labs serving Unguja and Pemba traveled to Bagamoyo to train with Sarah, Tunu, Zena, Grace, Happy and the IHI Bagamoyo lab scientists and were equipped with the knowledge and skills for SARS-CoV-2 testing and detection of other viruses of pandemic potential, a critical element in effective test and trace strategies for disease control.

International cooperation for pandemic response

Additionally, in response to a request from the Government of Equatorial Guinea, IHI's team prepared scientists to support labs with SARS-CoV-2 detection, response, and control efforts. The IHI lab conducted an intensive training for four interns and one lab scientist (four male and one female) and deployed this team to Equatorial Guinea where they are now actively testing samples for SARS-CoV-2.

In Tanzania and beyond, women are often underrepresented in leadership and technical roles in the sciences. But at both of PREDICT's partner labs in Tanzania, women have been core to the disease detection workforce. In Bagamoyo, under Grace's mentorship, a new cadre of female leaders has been empowered and placed in key positions to support national health security. These women, some of whom started as students and in intern positions at PREDICT's SUA lab, are the current and future workforce standing between Tanzanians and the biological forces that threaten their health and livelihoods.

PHOTO: PREDICT TANZANIA

ABOUT THIS STORY

For over 10 years (2009-2020), the USAID PREDICT project strengthened national laboratory capacity in Tanzania using the One Health approach. PREDICT partner labs at the Sokoine University of Agriculture and Ifakara Health Institute were trained and equipped in the full range of activities required for safely detecting zoonotic viruses, including biosafety and biosecurity, cold chain, safe specimen storage, data management, safe specimen transport and shipping, and molecular virus detection techniques. As a result, both labs are now recognized by the National Public Health Laboratory and National One Health Platform as centers of excellence for the safe detection of priority zoonotic diseases (SARS and MERS coronaviruses, Ebola and Marburg, Rift Valley Fever, and zoonotic influenza viruses) as well as emerging threats such as "Disease X", listed in WHO's blueprint for research on priority diseases. These labs also serve as key training centers for students and professionals, including government staff from the national lab system, and have been instrumental in supporting the health sector's journey to self reliance.

LEARN MORE

From 2009-2020, PREDICT's One Health laboratory team in Tanzania provided expertise and knowledge to the national laboratory system through hands-on training including One Health short-courses in disease detection.

Read more at **p2.predict.global**.

ONE HEALTH PARTNERSHIPS & POLICIES

Moving beyond individual and institutional capacity building, PREDICT teams often had critical involvement in country-level coordination and policy development processes, particularly with regard to awareness of One Health approaches and raising key issues often absent from routine discussions (e.g. pathogen spillover from wildlife). As countries were at different stages of their formal One Health coordination, from no existing coordination mechanism to well-established platforms, these approaches were appropriately targeted to country context. Local PREDICT team members participated in major assessment and planning processes (e.g. Action Plans). These activities reinforced global-level efforts to refine assessment and planning tools and processes to better account for emerging infectious disease spillover risks and mitigation strategies.

ONE HEALTH PLATFORM DEVELOPMENT

8

- ETHIOPIA
- GHANA
- GUINEA
- JORDAN
- LIBERIA
- NEPAL
- RWANDA
- SIERRA LEONE

JOINT EXTERNAL EVALUATION

8

- BANGLADESH
- CÔTE D'IVOIRE
- INDONESIA
- KENYA
- SENEGAL
- SIERRA LEONE
- TANZANIA
- THAILAND

AMR NATIONAL ACTION PLANS, NATIONAL BIODIVERSITY STRATEGY & ACTION PLAN, NATIONAL ACTION PLAN FOR HEALTH SECURITY

4

- INDIA
- INDONESIA
- LIBERIA
- TANZANIA

AHSC
APHA
BLUE RIBBON STUDY PANEL ON BIODEFENSE
World Health Organization
THE WORLD BANK
Food and Agriculture Organization of the United Nations
UNISDR
Convention on Biological Diversity
CITES
OIE
GHSAC

DURABLE IMPACT

PREDICT's work also helped shape systematic changes that will make early warning and detection of disease threats from wildlife routine in national and subnational preparedness. Through national mandates and improvements in domestic financing and new regulations, PREDICT is leaving a durable impact on national structures and systems, demonstrating how improved awareness and information on zoonotic pathogen risks from wildlife can help identify gaps and offer opportunities to tackle known and novel threats.

NEW MANDATES

In Ghana, wildlife disease and zoonotic disease prevention, control, and response are now included as activities in the strategic plans for the Veterinary Services Department as mandated by the National government, making a position of Director for Wildlife Diseases in the new Veterinary Services Act to acknowledge the relationship between wildlife and emerging diseases. In Bangladesh, the National One Health Secretariat has formed as a high level partnership across the Ministry of Health, Department of Livestock Services, Forestry Department, and laboratory network.

DOMESTIC FINANCING & REGULATIONS

With the discovery of filoviruses (the new Bombali virus and Marburg virus) by PREDICT-2 in Sierra Leone, the Guinean Ministry of Health intensified virus surveillance by allocating more material and financial assistance to their main Viral Hemorrhagic Fever laboratories. Given zoonotic disease risks, there are now regulations on hunted animals for consumption, public health requirements for the inspection of wild animal meat sold at markets, referral of cases of any type of hemorrhagic fevers to the district health office, and risk communication campaigns for social and behavioral change.

INTERNATIONAL PLATFORM ENGAGEMENT

PREDICT identified several areas of need and opportunity for international policy change to shift from reactive, resource-intensive responses to improved preparedness frameworks. Outputs from PREDICT activities included operational guidance, evidence syntheses, and policy changes.

OPERATIONAL GUIDANCE

PREDICT served as a technical expert resource for a wide range of partners at national, regional, and global levels in the development of guidance, sharing operational examples and lessons learned, and identifying operational gaps. Key documents included:

World Bank Operational Framework for Strengthening Human, Animal, and Environmental Public Health Systems at their Interface

PREDICT-2 contributed significantly in the technical development and coordination of the World Bank's "One Health" Operational Framework published in 2018, a compendium for One Health to date and a basis for understanding and implementing a One Health approach in global and country investments. Over twelve thousand copies of the document have been downloaded or distributed to date. It examines the strategic context, rationale, and case for investing in One Health; provides an inventory of tools and other resources from the human health, agriculture, environment, and disaster risk reduction sectors to facilitate multi-sectoral coordination; and includes technical guidance for appropriate One Health entry points and operations along the prevent-detect-respond-recover spectrum of preparedness. Specific examples and evidence base from the PREDICT project are provided. As a key tool with guidance included specifically for World Bank task teams responsible for project development and oversight, the Framework serves as a key resource for the World Bank's $300 million-plus Regional Disease Surveillance System Enhancement ("REDISSE") investment in West and Central Africa.

PREDICT disseminated the Operational Framework to a range of development donors and technical partners. A key success was in delivering the first Operationalizing One Health workshop in Côte d'Ivoire, which distilled down the Operational Framework to key topics and guidance of relevance for national implementation.

Through a focus on applied exercises, seven government ministries (including health, finance and economics, and rural development) identified practical approaches for increasing routine coordination in preparedness and capacity strengthening initiatives. Wildlife disease was highlighted as an area of technical capacity need, later followed up with a field-based wildlife health training for officials in Côte d'Ivoire held by the PREDICT Liberia team. PREDICT also led a paper with partners from the World Bank, WHO, CDC, FAO, OIE, Toward a Safer World Network, and the EPT-2 OHW and Preparedness and Response (P&R) projects to show opportunities for alignment of assessment and planning tools ("Institutionalizing One Health: from Assessment to Action", Health Security 2018).

World Health Organization (WHO)

PREDICT served as an expert for the FAO/OIE/WHO Tripartite Zoonoses Guide on "Taking a multisectoral, one health approach: a tripartite guide to addressing zoonotic diseases in countries" published in March 2019. The Guide features approaches shared by PREDICT, particularly for upstream risk reduction and the economic rationale for investing in One Health, and refers to several policy and evaluation guidance resources produced by PREDICT. Collaborations on tools and meetings were championed through the WHO Strategic Partnership for Health Security, including the first Joint External Evaluation mission and the Guide for Multisectoral Partnership Coordination for IHR and Health Security.

World Organisation for Animal Health (OIE)

PREDICT was a critical source of information to the OIE, via the OIE Working Group on Wildlife, expert meetings on MERS and ongoing reporting of emerging or noteworthy wildlife and disease issues. Directly reaching Chief Veterinary Officers through invited presentations to the OIE Assembly, PREDICT elevated the importance of including wildlife considerations as part of animal and public health efforts.

Convention on the International Trade of Endangered Species of Wild Fauna and Flora (CITES)

Given the importance of efficient investigations of major infectious disease events for health security, PREDICT-2 has liaised with international animal trade organizations to address permitting delays that have hindered investigation of recent wildlife mass mortality events. PREDICT personnel were appointed to serve on a working group on simplified procedures to promote timely movement of emergency diagnostic specimens convened by countries under CITES. The working group issued recommendations including permit exemptions for disease emergencies, ultimately approved in September 2019, and allowing for expedited procedures for registered laboratories. These actions represent critical steps forward to improve outbreak response.

Convention on Biological Diversity (CBD)

PREDICT provided key input in the landmark WHO-CBD State of Knowledge Review ("Connecting Global Priorities: Biodiversity and Human Health"), serving as lead coordinating author of the infectious disease chapter, where targeted risk management information was provided to enable work upstream of disease outbreaks. This report has served as a basis for ongoing technical input to the CBD as part of the Joint Work Programme with WHO (including PREDICT materials posted on the website) and as experts to the CBD-WHO Interagency Liaison Group.

PREDICT conducted training in Liberia on One Health policy and evaluation, leading to a draft intervention ultimately presented at the plenary at the CBD 14th Conference of the Parties. This intervention helped inform the decision on Health and Biodiversity accepted by Parties, which includes a recommendation for governments and other stakeholders to "review, adjust, and improve biodiversity-health linkages in the environmental assessment of relevant projects". This work was also paired with the detailed CBD Biodiversity-inclusive One Health Guidance, which emphasized early warning systems and attention to drivers of disease emergence.

Global Health Security Agenda (GHSA)

PREDICT-2 had a direct role in shaping the GHSA Roadmap 2024 which set out annual implementation and outcome objectives for the second phase of the GHSA, enhancing attention to multisectoral coordination and inclusion of the environment sector. As part of this engagement, PREDICT has had representation on the delegation representing NGO stakeholders (the Global Health Security Agenda Consortium) at GHSA Steering Group meetings. PREDICT also helped to develop the work plan for the GHSA Advocacy and Communications Task Force that will oversee information compilation for awareness of GHSA target outcomes and illustrates the value of continued prioritization of health security.

Along with sharing strategies deployed under the project, PREDICT team members had a direct role in development of other notable outputs such as:

- The first One Health policy statement under the American Public Health Association (APHA), which provided action steps from national to local level, including for integrating veterinary public health information in early warning systems
- Comprehensive cataloging of One Health core competencies covered by existing global curricula
- The Checklist for One Health Epidemiological Reporting of Evidence (COHERE) expert review group, which encouraged inclusive design and reporting on One Health-relevant research and interventions
- Indicators under the Global Health Security Index, including on policies for land use planning and animal disease management
- Presentations and expert meetings at Chatham House, the Gates Global Health Summit, the Consortium of Universities for Global Health, and the U.S. interagency Pandemic Prediction and Forecasting Science and Technology working group
- Technical advisor roles on the US Bipartisan Commission on Biodefense that identified needs for filling gaps in biodefense strategy and provided guidance for national strategic plans released in 2018 and 2019

PRODUCTS

Identifying several awareness and implementation gaps, PREDICT developed public tools and overview briefs for policy makers and technical partners to address key themes for One Health operations and value generation. Some information products were also developed in partnership with the P&R project to assist national One Health coordination platforms and other stakeholders (e.g. the private sector). All emphasized practical starting points, creating initial guidance or distilling a theoretical literature base into practical approaches. Examples include:

- Quick Guide to One Health Evaluation: targeted to serving needs of decision makers weighing how to best meet objectives and allocate resources
- Role of the Environment in Health Security and One Health: an overview of high-yield roles for environmental authorities to address emerging infectious disease (EID) risks, intended as an entry point to improve inclusion of the environmental sector in national and sub-national plans and operations and cooperation with public and animal health authorities
- Audit tool and checklist for extractive industries: to encourage voluntary uptake of risk reduction practices by the private sector; P&R and PREDICT developed tools for assessing potential EID impacts from project design, implementation, and evaluation, given that impact assessment for emerging infectious diseases are not routinely conducted for economic development projects. These were reviewed by private industry for alignment with their processes, and they build on existing environmental and social safeguard frameworks to demonstrate feasibility.

REFLECTING ON THE PAST & LOOKING FORWARD

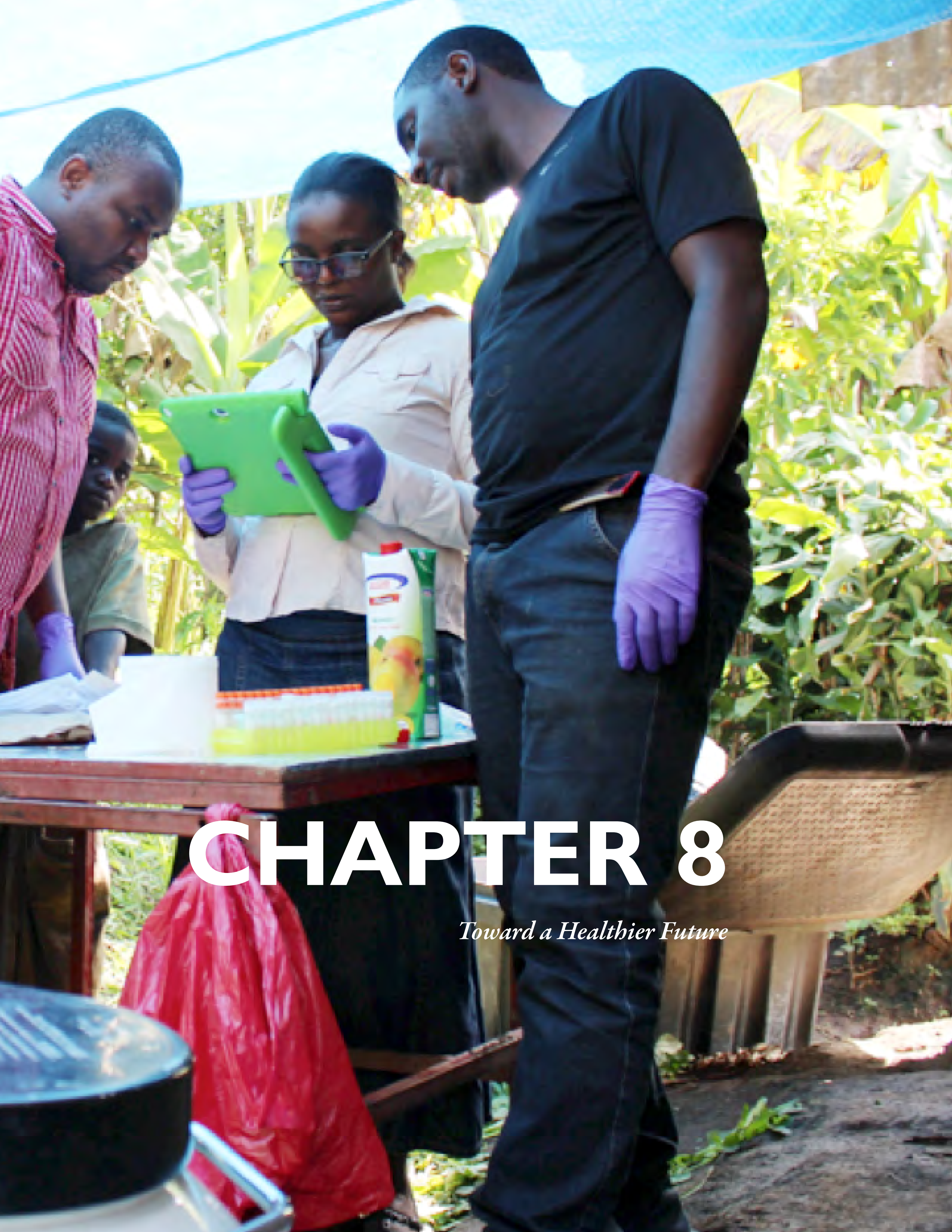
As the world scrambled to respond to the earliest stages of the COVID-19 pandemic, the PREDICT legacy resources, including field guides, data, and trained scientists and technicians at more than 60 national, university, and partner laboratories were among the best response resources available to assist with detection and control of COVID-19 around the world. These workers, institutions, structures, networks, and systems are ready and waiting to respond to future emerging viral threats with the same level of commitment, energy, and technical capacity as they've responded to one of civilizations' greatest challenges in living memory.

There is no doubt that PREDICT contributed to the transformation of how some laboratories and national health systems address health security. In all of the countries where PREDICT worked, standardized laboratory protocols, a universal testing platform that works across all mammalian species, and a cadre of One Health workers and advocates are contributing to improved global health security. Improvements in basic infrastructure such as backup power, cold chain capacity, and molecular diagnostics should not be overlooked as major contributions to institutional capacity that will long outlive the project. But even more enduring are the thousands of individuals inspired and enabled to work across sectors and champion the One Health approach. With over a decade of experience engaging in numerous countries around the world, the PREDICT project successfully trained, supported, and networked over 6,000 wildlife, veterinary, and human health professionals at the local, subnational, and national levels to facilitate knowledge sharing and ongoing working relationships that break down the historical barriers among sectors and disciplines. Reiterated time and again from voices in the community and stakeholders across levels was the value and appreciation of consistent community engagement across all stages of the project. The power of the One Health approach lies in the trust and innovation that can emerge from such collaborative team efforts to address complex challenges, such as zoonotic viral spillover. The beauty of the One Health approach is the commitment and enthusiasm shown by so many of those trained to pass on their knowledge and build up One Health competencies in the next generation of students, researchers, health care professionals, front line workers, and policymakers.

PREDICT has catalyzed positive change for development and instilled best practices for world class science and disease surveillance in every country in which it has worked. Resulting from standardized protocols and approaches for conducting concurrent One Health surveillance in animal and human populations, project findings were designed to be comparable across study sites, countries, and regions, and this comparability has led to breakthrough discoveries of what is and isn't known to be present in wildlife populations. There is much more work to be done to catalogue and characterize the known and novel pathogens present in wildlife that pose public health threats, building upon the capacity strengthening lessons learned and successes to date. The value and necessity of multisectoral involvement for health security, and particularly biothreat detection and prevention, is now obvious. It is still the case that wildlife surveillance is underpowered, in human resources and in funding base, compared to domestic animal and public health surveillance systems around the world, and this obstacle can only be overcome if the importance and commitment to understanding wildlife as reservoirs of potentially pandemic viruses is shored up in order to prioritize wildlife work as critical to reducing the future global burden of disease, with SARS-CoV-2 as a clarion call to action.

> “PREDICT has catalyzed positive change for development and instilled best practices for world class science and disease surveillance.”

PHOTO: PREDICT TANZANIA



CHAPTER 8

Toward a Healthier Future

PHOTO:: NIGEL WALKER

CLOSING THE KNOWLEDGE GAP

PHOTO:: PREDICT TANZANIA

Global trends indicate that over the course of this century, the rate that new microbial threats emerge will continue to accelerate due to growing socioeconomic and demographic factors, largely driven by the world's increasing population and interactions with animals. Success in preventing pandemics and the uncontrolled spread of epidemics requires thinking and acting differently. Revolutionary advances in health science and technology have made it possible to close the knowledge gap and discover what viral threats exist.

The wealth of information collected by the PREDICT Consortium should have lasting impacts on the world well beyond the timescale of the project. We identified high-risk human behaviors at interfaces facilitating viral transmission, as well as the human-induced drivers for pathogen emergence. Through demonstrated products and data repositories, PREDICT has provided a vast resource of new, open access material for integration into future epidemiological investigations and pandemic prevention efforts. By creating novel, easy to use tools, including the PREDICT HealthMap site and SpillOver online risk ranking tool, the PREDICT team has, for the first time, created innovative resources for policy makers to use in order to make scientifically-informed decisions when assessing the public health risk of novel viruses. Our project has provided the foundation and laid the groundwork for future development and better understanding of the risks posed by novel viruses of wildlife origin.

We provided the structure and evidence base for scaled-up efforts to prevent and prepare for future pandemic events. Our data, protocols, and informed and engaged approaches are informing major international initiatives, including a Pandemic Interception System (Kress et al., 2020), the Trinity Challenge (**https://thetrinitychallenge.org**), and the Global Virome Project (Carroll et al., 2018).

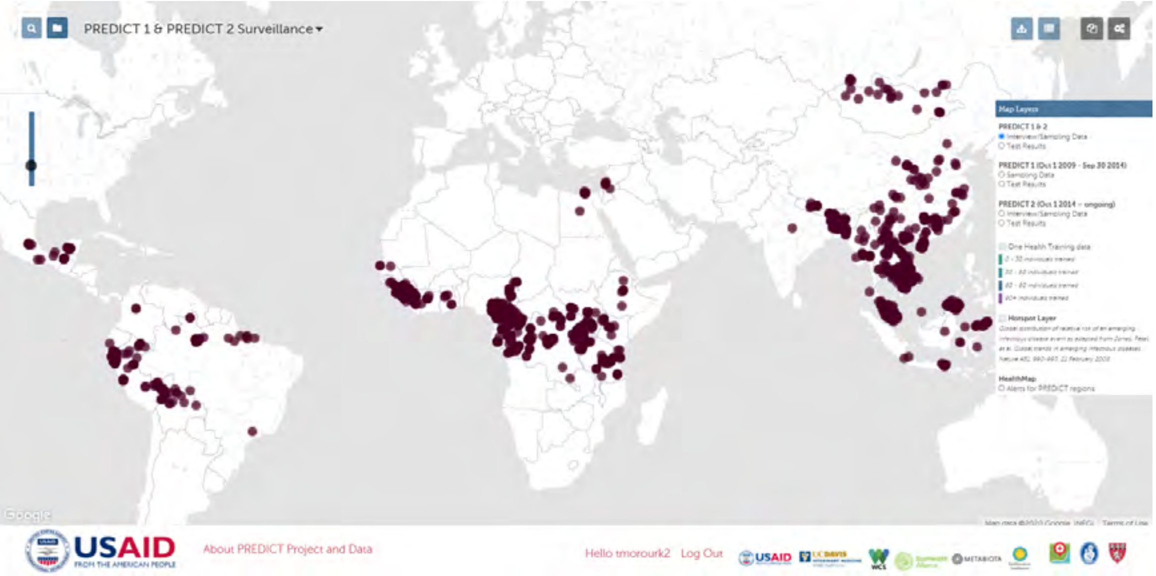


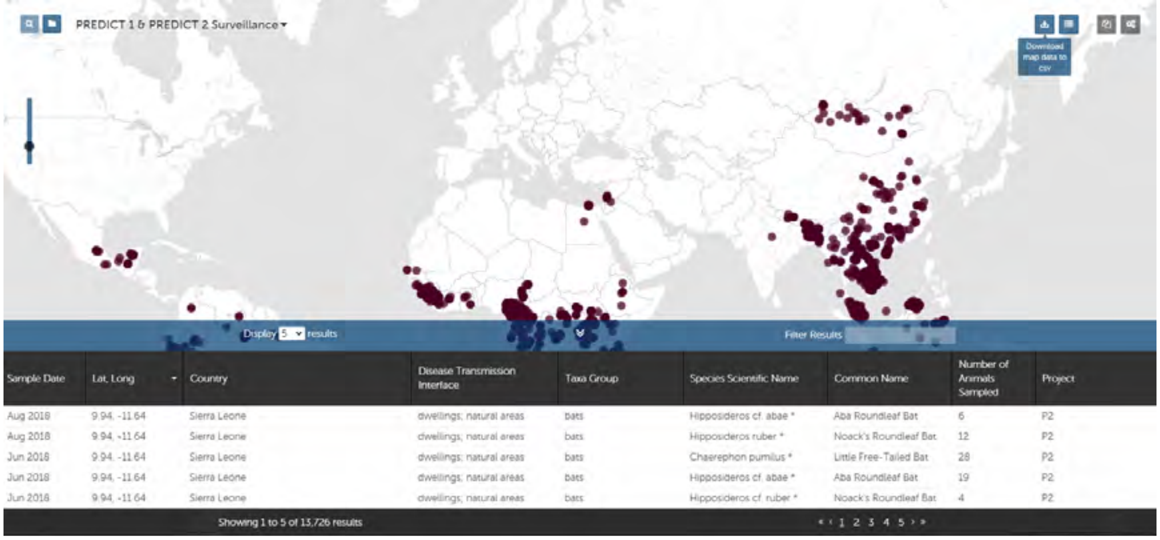
FIGURE 1. *The PREDICT HealthMap website interface* (**www.healthmap.org/predict**)

THE PREDICT HEALTHMAP SITE

HealthMap is a machine learning tool that aggregates informal online sources, including news media and social media posts, from around the globe to track infectious disease outbreaks 24/7/365 in 15 functional languages. This platform highlights disease trends globally, providing useful insights to inform public health decision making. Freely accessible, HealthMap is used by highly recognized government institutions, including .the US Centers for Disease Control and Prevention, US Department of Health and Human Services, US Department of Defense, the World Health Organization, as well as public health institutions and the public. The site receives over 1,000,000 visits per year.

The PREDICT HealthMap site (**www.healthmap.org/predict**) is an open access section of the HealthMap platform that allows users to visualize the PREDICT data on an interactive world map (Figure 1). By hovering over a datapoint, users can view aggregated PREDICT sampling data and virus test results for each location in all of the PREDICT-engaged countries. Detailed data extracts, including information about the animals, locations, and interfaces investigated, are available if the user creates a free HealthMap account (Figure 2), and data can be downloaded freely directly from the site.

The tool provides the option to overlay the PREDICT data with other global emerging infectious disease data sources, including a map of areas predicted to be hotspots for the emergence of infectious diseases (Jones et al., 2008), as well as HealthMap infectious disease alerts. Disease alerts are real-time reports that bring together disparate data sources, including online news, eyewitness reports, expert-curated discussions, and validated official reports. This function allows quick identification and integration of PREDICT data into the public domain should a PREDICT virus become an emerging health issue.



Sample Date	Lat, Long	Country	Disease Transmission Interface	Taxa Group	Species Scientific Name	Common Name	Number of Animals Sampled	Project
Aug 2018	9.94, -11.64	Sierra Leone	dwellings; natural areas	bats	Hipposideros cf. abae *	Aba Roundleaf Bat	6	P2
Aug 2018	9.94, -11.64	Sierra Leone	dwellings; natural areas	bats	Hipposideros ruber *	Noack's Roundleaf Bat	12	P2
Jun 2018	9.94, -11.64	Sierra Leone	dwellings; natural areas	bats	Chaerephon pumilus *	Little Free-Tailed Bat	28	P2
Jun 2018	9.94, -11.64	Sierra Leone	dwellings; natural areas	bats	Hipposideros cf. abae *	Aba Roundleaf Bat	19	P2
Jun 2018	9.94, -11.64	Sierra Leone	dwellings; natural areas	bats	Hipposideros cf. ruber *	Noack's Roundleaf Bat	4	P2

Showing 1 to 5 of 13,726 results

FIGURE 2. ***Example of a detailed PREDICT HealthMap data extract***

THE PREDICT DATA DEVELOPMENT LIBRARY

The PREDICT-2 data (2014-2020) has been made publicly available through the PREDICT Emerging Pandemic Threats Project Data Asset in the new USAID Data Development Library. Access to the data asset has been provided to the public by USAID without any restrictions at **data.usaid.gov**. The data asset consists of multiple datasets describing:

- The location where animals or people were sampled (**Site / Event Characterization**)
- The demographics of animals or people sampled (**Animals Sampled / Humans Interviewed**)
- The animal or human specimen virus test results (**PCR Tests**)

DESCRIPTION OF THE DATA

The **Site / Event Characterization dataset** describes the location, characteristics, and observations made during a human or animal surveillance event. Additional surveys (modules) were completed to describe the high-risk disease transmission interface(s) present at each event. Disease transmission interfaces include: animal production, crop production, human dwellings, ecotourism, and animal value chains, among others.

The **Animals Sampled dataset** contains the biological information, such as the species, age, and sex of the individual animals captured and sampled during a surveillance event.

The **Humans Interviewed dataset** provides responses to questionnaires conducted in locations concurrent to where animals were sampled. People were recruited from both syndromic (hospital/clinic) and community settings. In order to protect the privacy of respondents, data are displayed aggregated by country, district, gender, and year of interview. Each person completed surveys relating to their demographics, medical history, animal contact, and livelihood. If the participant worked in a high-risk occupation, additional survey modules were conducted to provide further insight into activities and behaviors that might put the participant at risk of zoonotic viral transmission. If the interviewee was unwell or residing in a clinic or hospital at the time of the interview, questionnaires were completed to describe the disease symptoms and other relevant information.

The **PCR Test Result dataset** contains descriptions of the samples collected from animals and people during surveillance activities. Several PCR tests for different virus groups were performed on each sample. The dataset includes all test results, positive and negative. For samples that tested positive by PCR, the virus name, interpretations, genetic sequence, and Genbank Accession numbers were provided.

Scan with your iPhone or other code reader to access the PREDICT data at USAID's data development library

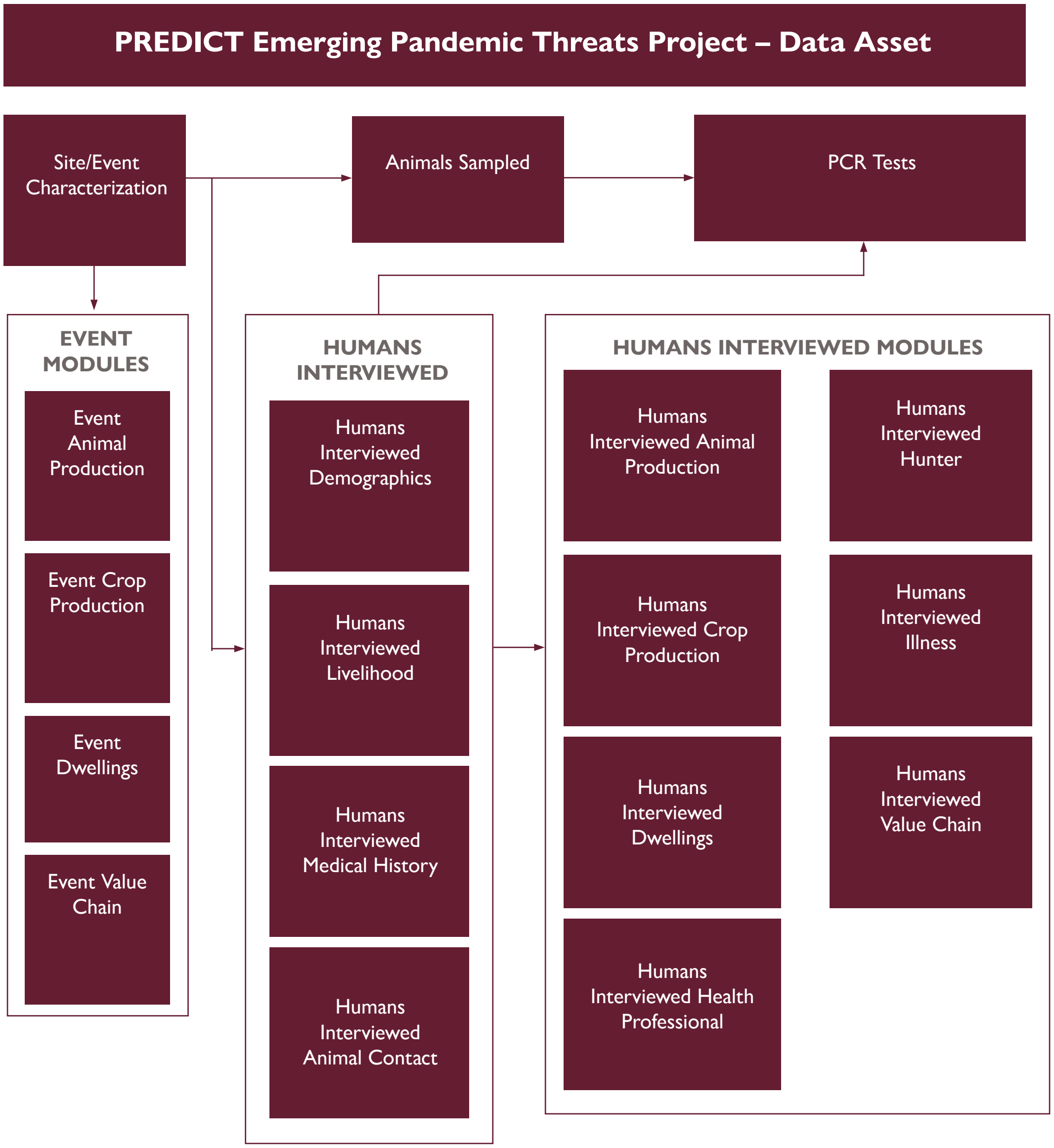


FIGURE 3. *PREDICT-2 data asset structure showing relationships among datasets and how they are linked*

PHOTO:: DAVID McIVER

SPILLOVER: VIRAL RISK RANKING

A NEW TOOL FOR RANKING THE RISK OF VIRAL SPILLOVER

Throughout human history, zoonotic viruses have been responsible for major pandemics, with devasting social and economic consequences. There are just over **250 known zoonotic viruses** – viruses that have previously spilled over from animals to humans and caused disease in people (Carroll et al., 2018). The bigger concern, as the recent SARS-CoV-2 pandemic demonstrates, is that ***unknown*** viral threats can emerge at any time.

It has long been predicted that emerging infectious diseases (EIDs), particularly zoonotic viruses, would cause the next pandemic (Jones et al., 2008). Our scientists estimated that there are **more than 500,000 animal viruses that have the potential to spillover** from animal sources to humans. We know very little, if anything, about these viruses, but the risk each poses is not equal. Several factors about the virus and the associated host, environment, and human behavior influence the likelihood that a virus will spillover and cause a pandemic.

Virus discovery efforts, of which the PREDICT project is a leading example, can create an essential catalog and rapidly expand our knowledge of what viral threats are out there. However, without a strategy to evaluate risk and rank and identify the most important viruses, we are faced with a lot of data and little direction upon which to focus our efforts for preventing future pandemics.

To address this problem, the PREDICT team developed a new tool to systematically evaluate novel wildlife viruses in terms of their zoonotic spillover and pandemic potential.

SNAPSHOT

- **SpillOver** is the first comprehensive open-source risk assessment tool that evaluates animal viruses in terms of their zoonotic spillover and pandemic potential.
- **SpillOver** uses the concept of a **'Credit Report'** – using key factors to assess risk and rank viruses to identify those of greatest potential threat to human health.
- By creating a **watchlist of viruses**, we can prioritize targets for vaccines, therapeutics, and intervention measures, which taken together could result in pandemic ***prevention*** for a fraction of the cost of pandemic ***response***.

> "To prevent future pandemics we need to conduct and communicate science in a collaborative and transparent way. The open-source SpillOver tool is designed to be adaptive – the more data we get, the better our risk estimations – and the closer we come to accurately predicting spillover potential. We can prevent the next pandemic by working together."
>
> —Dr. Zoë Grange

BUILDING THE SPILLOVER TOOL

SPILLOVER
VIRAL RISK RANKING

FIGURE 4. ***Components of the SpillOver tool***

Using literature reviews and input from leading One Health scientists with both field and laboratory expertise, we identified 33 risk factors which are thought to have the most influence on viral spillover and spread in human populations (see below for examples). We then created a webtool called **SpillOver: Viral Risk Ranking** (**https://spillover.global/**) that uses these risk factors and a substantial database, as well as data from multiple publicly available datasets, to calculate a comparative 'spillover risk score' for each animal virus, much like a credit report.

SpillOver produces a detailed spillover risk report for each virus, and the **'Risk Comparison' tool allows users to compare and contrast ranked viruses**, as well as filter viruses on a selection of key attributes, including virus species, host species, and country of detection. Scientists are also able to **contribute data to existing viruses or assess the spillover and spread risk of new viruses** using the 'Rank Your Virus' application (Figure 5).

The PREDICT project detected hundreds of animal viruses at high-risk disease transmission interfaces around the world. Although these new viruses were from virus groups of concern to human health, their individual zoonotic spillover potentials were unknown. We used the tool to rank the spillover and spread risk of viruses detected by the PREDICT Consortium and compared them to viruses that were already known to be zoonotic. As of September 2020, the SpillOver tool has ranked 887 animal viruses from 19 virus groups. The majority of top-ranked (i.e. greatest risk for spillover) were known zoonotic viruses, as expected, since these zoonotic viruses have already spilled over from animals to humans. Interestingly, the tool identified several newly-discovered coronaviruses which are not currently known to be zoonotic as higher risk for spillover and spread than some known zoonotic viruses (i.e. have already had a spillover event). This result illuminates the potential power of the SpillOver tool, suggesting these newly discovered coronaviruses should be included in a watchlist for further investigation (Table 1).

TABLE 1. ***The highest ranking viruses in terms of spillover risk that were detected during the PREDICT project. These viruses are not currently known to be zoonotic.***

Ranking Position Among All Zoonotic Viruses	Virus Family	Virus Genus	Virus
20	Coronaviridae	Betacoronavirus	SARS-related betacoronavirus Rp3
24	Coronaviridae	Alphacoronavirus	Coronavirus 229E (Bat strain)
28	Coronaviridae	Betacoronavirus	Murine coronavirus
29	Coronaviridae	Betacoronavirus	Rousettus bat coronavirus HKU9
31	Coronaviridae	Alphacoronavirus	Chaerephon bat coronavirus/Kenya/KY22/2006
32	Coronaviridae	Alphacoronavirus	Coronavirus PREDICT CoV-35*
33	Coronaviridae	Betacoronavirus	Longquan Aa mouse coronavirus
36	Coronaviridae	Betacoronavirus	Eidolon bat coronavirus/Kenya/KY24/2006
37	Coronaviridae	Betacoronavirus	Coronavirus PREDICT CoV-24*
38	Adenoviridae	Mastadenovirus	Human mastadenovirus G
39	Coronaviridae	Betacoronavirus	Coronavirus HKU1
40	Coronaviridae	Betacoronavirus	SARS-related bat coronavirus RsSHC014
41	Coronaviridae	Betacoronavirus	BtVs-BetaCoV/SC2013
42	Coronaviridae	Alphacoronavirus	Rodent coronavirus
43	Coronaviridae	Betacoronavirus	Coronavirus PREDICT CoV-22*
44	Retroviridae	Spumavirus	Macaque Foamy virus
46	Filoviridae	Ebolavirus	Bombali Virus*
47	Astroviridae	Mamastrovirus	Mamastrovirus 1
49	Reoviridae	Rotavirus	Rotavirus A
50	Coronaviridae	Betacoronavirus	Kenya bat coronavirus/BtKY56/BtKY55

*Novel virus first detected and identified during the PREDICT project

IMPLICATIONS

The power of this tool lies in the fact that it is **open-source** – the more data entered, the more robust the ranking. SARS CoV-2 currently does not rank as number one among known zoonotic viruses, which may seem counterintuitive given the global devastation the virus has caused. The problem is that prior to its emergence, we did not know the virus was there (we hadn't looked in that specific geographic region), and we still lack key data that would allow the SpillOver tool to accurately estimate SARS-CoV-2 spillover risk such as:

1. Number and range of host species,
2. Geographic distribution of its' hosts, and
3. Types of environments the hosts live in.

As we learn more about this virus, as could be accomplished through the Global Virome Project (see below), the higher SARS-CoV-2 will rank within the tool itself. That it does not currently rank higher demonstrates how critical it is for us to expand virus discovery research to increase our understanding of viruses, their hosts, and the processes that facilitate spillover.

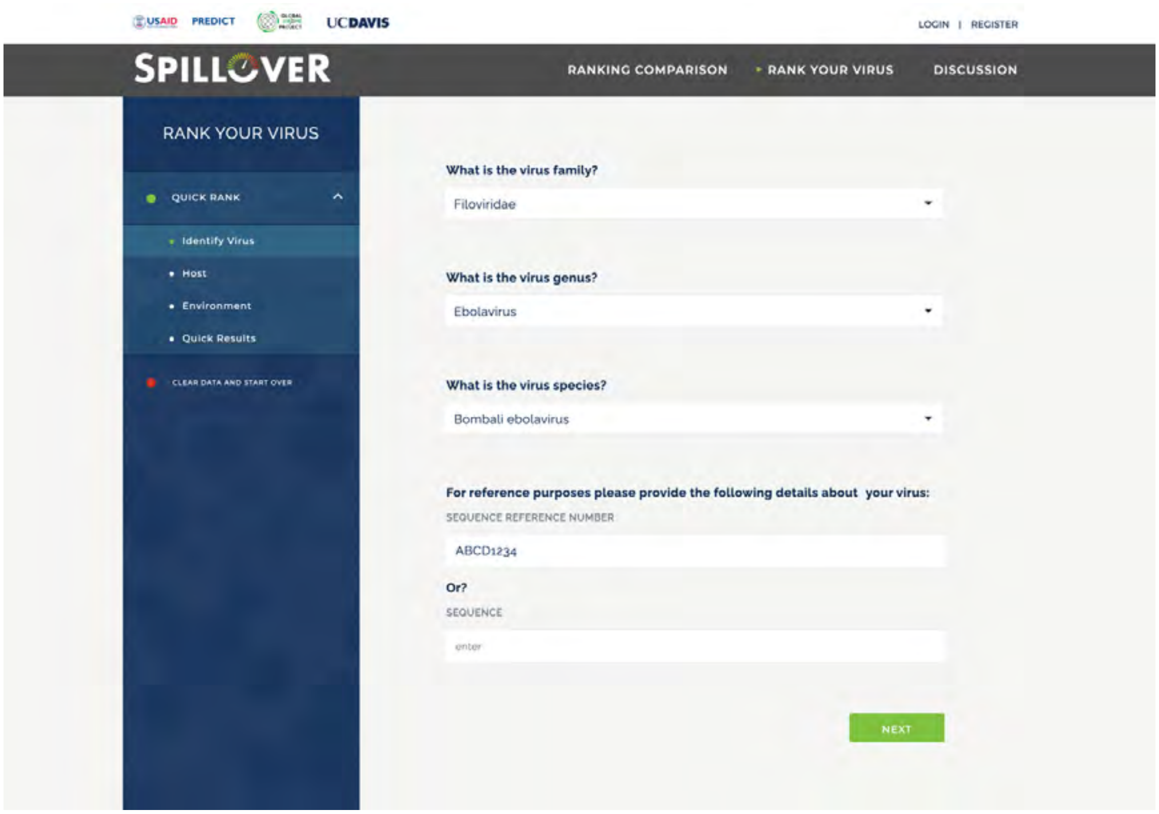
SpillOver is the **first-of-its-kind**, a dynamic risk assessment tool addressing the need for a globally-accessible platform that integrates, interprets, and ranks infectious disease data on a broader scale. PREDICT provided the **essential** initial dataset to build the tool and gain early insights into high-risk viral threats maintained in wildlife hosts. By then **crowd-sourcing and integrating virus discovery data from scientists around the world** into the SpillOver database, we will advance regional and global understanding of viruses and risk estimations for viral spillover and spread.

The SpillOver Viral Risk Ranking Tool is an essential first step in collating massive global data sets across multiple disciplines (virology, epidemiology, ecology, etc.), producing a meta-analysis that can focus research and vaccine development and ultimately inform public health interventions to reduce the likelihood of future pandemics.

TOP RISK FACTORS FOR VIRAL SPILLOVER

Ability of the virus to transmit between animals and humans, as well as spread human to human:

- Ability of the virus to infect humans
- The type, intimacy and frequency of interactions between wild/domestic animals and humans
- How the virus is transmitted between hosts
- Whether the virus has been implicated in epidemic/pandemic events
- The number and diversity of hosts the virus infects



What is the virus family?

Filoviridae

What is the virus genus?

Ebolavirus

What is the virus species?

Bombali ebolavirus

For reference purposes please provide the following details about your virus:

SEQUENCE REFERENCE NUMBER

ABCD1234

Or?

SEQUENCE

enter

FIGURE 5. *The SpillOver* **Rank Your Virus** *tool is a crowd-sourcing application that allows scientists to rank the spillover risk of new viruses and add information to the SpillOver database.*

We now live in an era in which threats posed by pandemics and epidemics are a global reality. A single lethal virus can emerge suddenly and spread rapidly to every household and every community without regard to national borders or to social and economic standing. The SARS, Ebola, and Zika outbreaks did little to prepare us for the COVID-19 pandemic sweeping the world and serves as a clarion call that we are vulnerable to emerging viral threats. Since the mid-20th century, new and deadly diseases have emerged at an alarming rate, and the threats from this vast pool of unknown viruses are accelerating exponentially, driven by our expanding population and global travel.

USAID's 11-year $200+ million PREDICT project investment provided the proof-of-concept that, if taken to scale, a Global Virome Project, identifying nearly all of the world's viral threats to human health, is feasible and within reach. We laid the foundation and determined the feasibility of such a globally-scaled project, as we built capacity to detect viruses in bats, rodents, and primates in 35 countries and discovered over 900 novel animal viruses with the potential to infect people, including a new Ebola virus in African bats. Through PREDICT, we also detected dangerous known pathogens in new regions and host species, expanding the knowledge available on risk of transmission for priority pathogens, such as Ebola Zaire, Marburg virus, and SARS-related coronaviruses, just the kind of data needed to make our SpillOver tool an incredibly useful resource for risk ranking. But the viruses we know are barely the tip of the iceberg. We estimated that there are more than 500,000 animal viruses, about which we know nothing or very little, that have the potential to spillover from animals to humans and cause disease (Carroll et al., 2018). Understanding these viral threats, then developing mitigation strategies to prevent infection and spread in people are key to stopping devastating future pandemics.

Building upon PREDICT's foundation, we hope the world will come together to use the latest tools to discover the majority of viral threats that can jump from wildlife sources to people. Initiatives, such as the Global Virome Project, represent a strategic response to the growing need to better predict, prevent, and respond to future viral pandemic threats and to protect us all from their worst consequences. One of PREDICT's most promising legacies is the proof of concept that virus discovery on a pandemic-averting scale is possible.

PHOTO: KATHY WEST

By bringing multi-disciplinary projects and interested parties together to share protocols and data, global networks can establish a joint coordinated effort, whereby countries will gain the benefits of shared global and regional strategies while maintaining autonomy to respond to local needs. Our vision is a world safe from the threat of emerging viral diseases. To better prepare for and stop future epidemics, we must develop innovative and productive partnership networks among public, private, philanthropic, and civil organizations to detect the majority of our planet's unknown viral threats to human health and food security.

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TOWARD A HEALTHIER FUTURE

We are at the precipice of a world safe from pandemic threats.

In the face of overwhelming uncertainty, we have the tools to prevent the next pandemic. Emerging infectious diseases are on the rise, but they do not have to define our near- or long-term future. For the scientific community, there is a clear roadmap with actions we must take. Through the PREDICT project, we have revisited decades of earlier research on virus diversity and evolution, wildlife host and disease ecology, and behavioral risk analyses that went without notice and whose warnings were not heeded. This wealth of available knowledge is helping public health officials understand and model scenarios for COVID-19 spread and informing coordinated response efforts. We have also set the foundation for essential systemic changes to increase nimbleness in our public health systems to respond efficiently in the future.

Our collaborative research has shed new light on global virus diversity, particularly for coronaviruses, and has given us incredibly valuable information on the roles of human behavior and wildlife host ecology in viral transmission patterns and future risk. For example, wildlife in markets and the human food value chain was specifically identified as a high-risk interface for spillover. Especially in the past decade, we have vastly increased our knowledge on the best approaches for detecting and discovering viruses of high pandemic potential that can move between animals and people.

Our team alone has discovered and characterized over 100 novel coronaviruses and contributed additional information for more than 60 that were previously known. PREDICT provided many of the tools we need to help reduce the risk of deadly spillover events and ensure pandemics like COVID-19 never happen again – we just need to use them. We have developed and field-tested cutting-edge approaches to reducing viral spillover in high-risk populations and pioneered methods of mathematically modeling viral spillover risk – powerful examples of the tools needed for the fight to prevent future pandemics. Fortunately today, those that have made the advancements above are serving as the One Health-trained workforce (scientists and policy makers who explore the intersection of planetary, animal, and human health). They are prepared for and pushing back the frontlines of COVID-19 in many countries around the world. We are on the precipice of a world safe from pandemic threats. We must use the lessons from PREDICT and take action, allowing science to inform our strategies and create a safer and healthier future for all.

In the face of overwhelming uncertainty, we have the tools to to prevent the next pandemic.

PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

PART TWO

“When ‘I’ is replaced with ‘We’ even illness becomes wellness.””

—Malcolm X (el-Hajj Malik el-Shabazz)

PHOTO: SIMON TOWNSLEY

PHOTO: ROSHAN PATEL, SMITHSONIAN'S NATIONAL ZOO & CONSERVATION BIOLOGY INSTITUTE

PREFACE

One Health in Action Around the World

INTRODUCTION

Emerging infectious diseases and pandemic threats are highly complex and dynamic global challenges. Science has shown us these challenges are driven in large part by an exponentially expanding global human population, human behaviors, and human activity. In today's globally connected reality there is no isolation from the threat of emerging disease, as microbes do not recognize boundaries. Individuals, communities, and countries are interdependent for knowledge, goods, and services from around the globe. The COVID-19 pandemic has proven that a disease outbreak from one country can quickly spread to other countries and regions, creating widespread and devastating impacts. SARS-CoV-2 is the latest in a long history of emerging viruses, and subsequent global pandemics are inevitable unless direct action is swiftly taken.

ONE HEALTH IN ACTION

Such dynamic and complex challenges require equally intricate solutions, and science shows us that it is key to collaborate across disciplines, cultures, countries, beliefs, and environments to understand emerging disease threats and to make positive impacts. One Health is the name put to the concept that human, animal, plant, and environmental health are inextricably connected and interdependent. Indeed, collaboration across diverse disciplines spanning human and veterinary medicine, conservation, and laboratory, environmental, economic, and social sciences is critical to understanding these complex challenges and in designing collaborative solutions. Since 2009, the PREDICT project has played a lead role in establishing One Health approaches globally and in formalizing national One Health platforms in partner countries. In over 30 countries, our teams have strengthened disease surveillance systems, provided early detection for diseases emerging from wildlife species, and built laboratory and diagnostic infrastructure, along with human resource capacity and know-how targeted directly at the sources of emerging disease threats.

Together with USAID, the PREDICT project has made substantive progress strengthening capacity to detect the vast diversity of viruses in high-risk wildlife reservoirs, facilitating a One Health approach to surveillance, and integrating One Health policy at national and subnational levels. Together with our in-country partners, PREDICT's discoveries and insights have led to a deeper understanding of the animal-human interfaces, geographies, communities, and behaviors that are high-risk for disease emergence, and we have developed the frameworks to put this knowledge into action towards outbreak detection, response, recovery, and prevention.

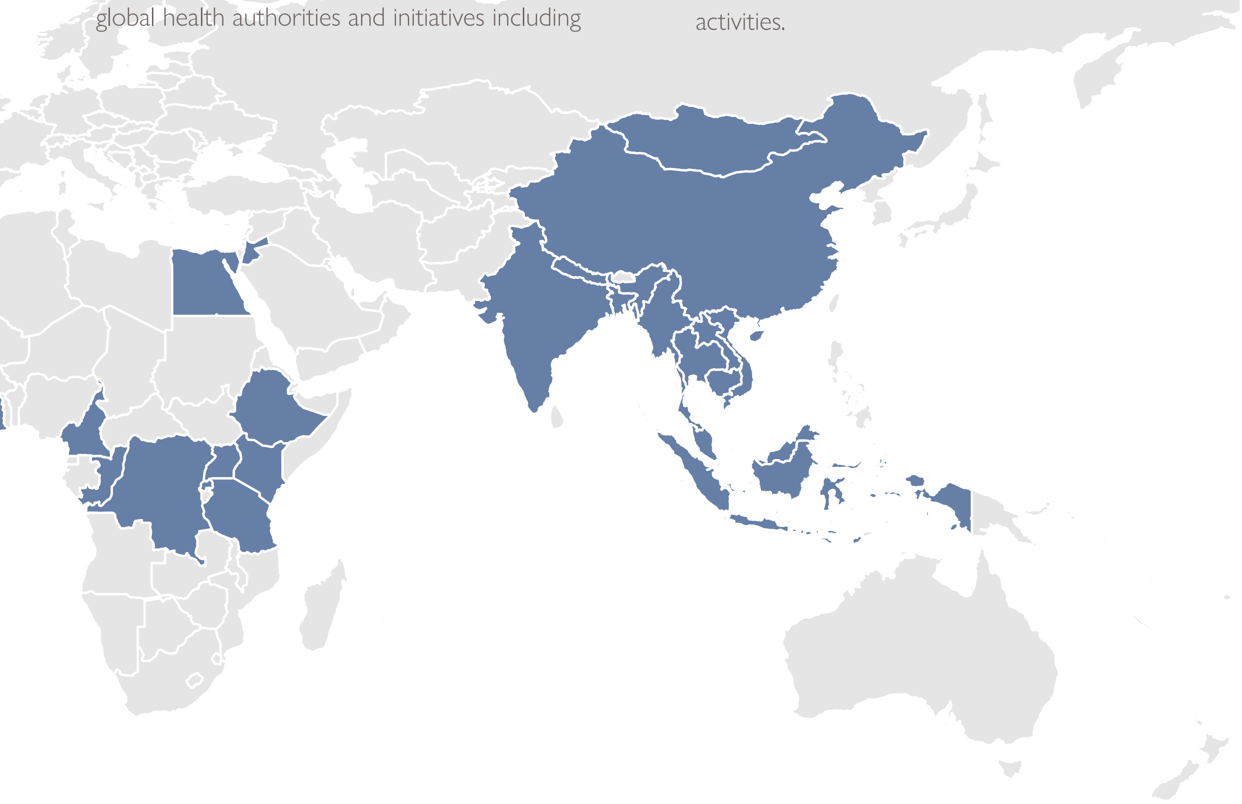
COUNTRY CHAPTERS

The PREDICT project's Country Chapters showcased in this volume represent the collaborative efforts of multi-disciplinary teams working together at community, national, regional, and global levels to establish and strengthen early detection, surveillance, and response systems in human and animal populations. In the pages that follow, you will meet the individuals who collectively made PREDICT into a successful project and read about their achievements, scientific discoveries, and contributions that are making their countries and our world a healthier place to live. PREDICT's country teams were the frontline workers in the field and laboratories, leading world class scientific efforts in remote locations, in the pursuit of generating robust data to inform globally oriented predictive systems that reduce future pandemic threats.

In many countries where PREDICT worked over the last 11 years, animal and human surveillance systems were informally linked and often activated on an ad-hoc basis to address an outbreak or health emergency. The PREDICT project's capacity building, training, and informed policy making efforts helped formalize these important linkages in the form of established national One Health platforms and strategic plans, connecting mandates across government ministries, communities, and academic and private sectors at the country level. These efforts were undertaken in alignment and partnership with global health authorities and initiatives including the World Health Organization (WHO), Food and Agriculture Organization (FAO), World Organization for Animal Health (OIE), and Global Health Security Agenda. Our project's success is marked by a strategic focus on strengthening systems and networks across human and animal surveillance systems. Our outputs have enabled stakeholders from community, national, and regional levels to establish multi-disciplinary One Health approaches that are led locally and nationally, and which have achieved important regional impacts.

UGANDA

One example is in Uganda, where our PREDICT team directly supported enhancement of national surveillance and laboratory systems, contributed to implementation of the country's Global Health Security Agenda roadmap, and strengthened mechanisms for responding to priority zoonotic diseases, including Ebola, Marburg, and Rift Valley hemorrhagic fevers. To better understand threats of wildlife pathogens to human health, we worked together with our in-country partners and targeted wildlife sampling efforts at critical interfaces where wildlife, domestic animals, and people have significant interactions. The project's collaborative efforts and engagement across public, academic, and private sectors supported the growth of Uganda's One Health workforce from the national to district level through field- and lab-based training and through collaborative surveillance and disease detection activities.



MONGOLIA

Located at the intersection of three major migratory bird flyways, our team in Mongolia enhanced zoonotic disease surveillance capacity for avian influenza viruses and established a real-world transboundary disease model for disease detection and response. Annual migratory movement of wild birds contributes to global movement of avian influenza viruses, which are a not only a major public health concern but also have significant economic impacts in the poultry and livestock sectors. In Mongolia, we strengthened diagnostic capacity at national and provincial laboratories and conducted annual trainings focused on wild bird identification, safe sample collection procedures, cold chain, sample storage, and PPE use, all critical for effective zoonotic disease surveillance systems and establishing heath security.

ONE HEALTH CASE STUDIES

PREDICT's One Health Case Studies provide excellent examples of One Health in action, illustrating real-world examples across a variety of countries and contexts, and highlighting the practical benefits of One Health strategies in both day-to-day and emergency response operations.

In Myanmar, for example, PREDICT helped build disease surveillance systems, strengthen community outreach, increase awareness of emerging infectious diseases, and facilitate establishment of a national One Health platform integrating the national wildlife authority for the first time. Together with our in-country partners, we were successful in bringing Myanmar's Ministry of Natural Resources and Environmental Conservation (MONREC) into national One Health implementation meetings, marking the first direct involvement of the environmental ministry in health policy consultations and as a primary contributor in development of the Myanmar's One Health National Strategic Plan (2017-2022). Leveraging laboratory and human resource capacity enhanced through the project, trained and well-informed health staff and community health workers can identify wildlife-human interface risk areas and potential at-risk

populations to target surveillance efforts and disseminate tailored risk communication information, promoting awareness of risk factors for zoonotic disease transmission within their daily lives.

OUR LEGACY

Around the world, the PREDICT project has provided proof of concept for the value of risk-based One Health approaches to emerging disease threats. Through engagement with communities, policy makers, and regional and global advisory bodies, our teams laid the framework for whole-of-society solutions motivated by local ownership and sensitivity to livelihoods, beliefs, culture, and gender. Quite literally, thousands of individuals came to the table, embraced a One Health approach to emerging disease surveillance, and worked together to set a new standard for collaborative research efforts on a global scale. This volume is dedicated to highlighting these massive efforts and accomplishments and showcasing our lessons learned, so that future efforts will continue to improve health locally while simultaneously contributing to global health security.

Our project's legacy is marked by global leadership in, and a deeper understanding of, the One Health approach which underpins USAID's Emerging Pandemic Threats program. This legacy will be sustained by the extensive networks of experienced professionals, comprising a skilled and prepared One Health workforce that continues collaborations with in-country partners and synergistic programs critical to preventing the next pandemic.

(Left-Right) PHOTOS: PREDICT UGANDA; PREDICT MONGOLIA; ROSHAN PATEL, SMITHSONIAN'S NATIONAL ZOO & CONSERVATION BIOLOGY INSTITUTE; EUNAH CHO PRESTON

PHOTO: PREDICT KENYA

AFRICA & THE MIDDLE EAST

PREDICT

CAMEROON

ONE HEALTH IN ACTION (2009-2020)

Using a One Health approach to strengthen disease surveillance to prevent zoonotic threats

Locally known as "Africa in miniature," Cameroon contains great ecological and cultural diversity. As Cameroon experiences rapid population growth, extractive industries such as mining and hydroelectric power are cutting into rainforests that are home to some of the world's largest populations of gorillas, chimps, and other apes. As industry and agricultural expansion into the forest continue to bring humans and animals into closer proximity, the risk of zoonotic disease emergence intensifies.

Using a One Health approach, the PREDICT team safely conducted human and animal biological sampling and human behavioral research to identify and characterize risk factors associated with zoonotic disease spillover. The team concurrently sampled non-human primates, bats, rodents and humans in selected villages and towns primarily in Southern Cameroon to explore the transmission dynamics of zoonotic pathogens within village communities and rural hospitals, and bushmeat markets to develop a deeper understanding of the behaviors, beliefs, and practices among people engaged in the bushmeat trade alongside active surveillance for zoonotic pathogens.

Through analysis of project data and findings, the PREDICT team was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

During the course of the PREDICT project, Cameroon experienced several disease outbreaks, including yellow fever and monkeypox, and when requested by the government of Cameroon, we assisted with field outbreak investigations. In addition, the team played a key role in activities under the Global Health Security Agenda, including assisting with evaluation of the country's strengths, gaps, and priority actions for enhancing national health security; prioritization of zoonoses of public health concern in Cameroon; and development of a national One Health policy.

IMPLEMENTING PARTNERS

- Ape Action Africa
- Centre Pasteur Cameroon
- Food and Agriculture Organization
- Metabiota Cameroon
- Ministry of Livestock, Fisheries and Animal Production
- Ministry of Forestry and Wildlife
- National Public Health Laboratory/Ministry of Public Health
- Ministry of Environmental Protection and Sustainable Development
- Ministry of Scientific Research and Innovation
- Ministry of Defense/Military Health Research Center
- Mosaic
- National Program for the Control and Fight Against Emerging and Re-emerging Zoonoses
- National Veterinary Laboratory of Cameroon
- One Health Workforce
- Preparedness Response
- University of Douala and the University of Maroua researchers
- United States Centers for Disease Control and Prevention

Global Health Security Agenda

>200

DEVELOPED the One Health Workforce by training more than 200 people in Cameroon.

>16.2K

OPERATIONALIZED One Health surveillance and sampled over 16.2K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Military Health Research Center

56,601
TESTS

118 PREDICT **25** KNOWN

DETECTED 143 unique viruses in both animal and human populations.

MOHAMED MOCTAR MOUCHIE MOULIOM

Country Coordinator, *Mosaic*

"PREDICT has effectively contributed to Cameroon's increased capacities for zoonotic disease surveillance and the detection of priority zoonotic diseases and unknown threats. The opportunity to work with specialists from various professional backgrounds and to share their experience has helped me to build my own capacity in the One Health approach and project management more efficiently than ever before."

VICTORINE MAPTUE TOGEUM

Human Clinical & Behavioral Research Coordinator, *Metabiota*

"The position I held in the PREDICT project has truly boosted my professional career. Indeed, the research carried out within the framework of this project enjoys great visibility and will be cited most often. This has helped increase my notoriety within the national and international scientific community. The Cameroonian government can turn the results of this research into concrete measures that build capacity in the health field."

ACHIEVEMENTS

- Through the PREDICT project's influence, Cameroon now has a growing team of experts at various levels of government and has formed the National Program for the Control and Fight Against Emerging and Re-emerging Zoonoses, partnering with many local institutions trained to assist in investigations of zoonotic disease outbreaks.
- The PREDICT team contributed to building the national One Health viral disease detection network for new and known zoonotic viruses at the Military Health Research Center (CRESAR) in Yaoundé, providing training opportunities to laboratory scientists and enhancing country capacity for disease detection.
- The PREDICT project worked with the Directorate of Veterinary Services of the Ministry of Livestock (MINEPIA) to develop training materials for zoonotic disease surveillance in wildlife for Ministry of Wildlife staff (eco-guards). These trainings were held during three national training sessions which cover twenty protected areas in Cameroon. This newly-constituted network aims to implement a wildlife event-based surveillance system to collect weekly health information on disease cases and mortality in protected areas.

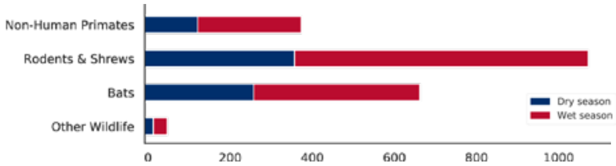
ONE HEALTH SURVEILLANCE

Through effective partnerships across animal and human health sectors and successful stakeholder engagement at national, regional, and local levels, the PREDICT project used the One Health approach for zoonotic disease surveillance. Increased bushmeat trade, combined with intensifying animal production and landscape changes due to activities such as hydroelectric dams and industrial rubber plantations, makes southern Cameroon a hotspot for high-risk interfaces between animals and humans. By integrating human biological surveillance and behavioral risk investigations with wildlife surveillance, focused in areas of increasing animal-human contact, the PREDICT team helped Cameroon to improve their capacity for disease detection, prevention, and response.

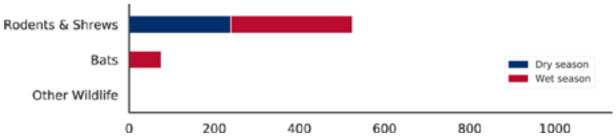
We conducted surveillance in two rural towns and at a bushmeat market, all located within the Dja et Lobo Division. These three areas in the South Region of Cameroon are located near the Dja Faunal Reserve, which is a UNESCO World Heritage Site with great biodiversity and is home to endangered species such as western lowland gorillas, chimpanzees, leopards, forest elephants, giant pangolins, bongo antelopes, and buffalos. Local populations hold traditional hunting rights around the Dja reserve, but over the past years, rubber projects implemented by foreign investors have converted 10,000 hectares of forest into rubber plantations (Corrie MacColl Cameroon Plantations), resulting in major social and environmental impacts. Rapid forest encroachment and land use change has been shown to lead to the emergence of infectious disease in wildlife (Daszak et al., 2001), and animals increasingly come into the villages to forage for food, exposing them to human populations.

We were particularly interested in enrolling people who exhibited symptoms that could be suggestive of exposure to viruses (such as influenza-like illness, severe acute respiratory infection, encephalitis, non-trauma related hemorrhage, or acute diarrhea), and especially those cases for which no other diagnosis could be made. Surveillance occurred at hospitals, communities, and wildlife markets. Biological sampling and syndromic surveillance of febrile patients occurred at two hospitals (one in each of the rural towns).

In these rural areas the primary source of hunted wildlife comes from the surrounding forests, thus wildlife hunters, transporters, sellers, and butchers were targeted for and enrolled in community-based surveillance. Individuals from crossroad markets, like the bushmeat market in a rural Dja et Lobo Division town where animals and meat (cooked or raw) are sold to the local public, were also enrolled in community-based surveillance. The people working in the animal value chain were interviewed about their wildlife interactions, and biological samples were also safely collected from animals they handle and work with, in order to determine the level of risk these practices might present.



Numbers of individuals sampled by taxa group in the town of Sangmelima and a bushmeat market in Dja et Lobo Division



Numbers of individuals sampled by taxa group at a rural town in Dja et Lobo Division

PARTICIPANT CHARACTERISTICS ACROSS HOSPITAL & SURVEILLANCE SITES

	COMMUNITY-BASED SURVEILLANCE	CLINIC-BASED SURVEILLANCE	
SITES	Sangmelima (n=215)	Sangmelima (n=218)	Town in Dja et Lobo Division(n=218)
GENDER (FEMALE)	67 (31%)	109 (50%)	108 (49%)
GENDER (MALE)	148 (69%)	109 (50%)	110 (51%)
CHILD (<18)*	7[#]	10 (2-17)	5 (2-17)
ADULT (≥18)*	44 (20-86)	38 (18-78)	37 (18-90)

**Average (range)*
[#] Sample size of 1

HIGHLIGHTS & FINDINGS FROM THE PREDICT PROJECT IN CAMEROON

- Confirmation of a monkeypox virus infection in primates as part of an outbreak response in a primate sanctuary, which was an important demonstration of ministerial, military, and CDC collaboration in Cameroon
- Detection of different bat coronaviruses, as well as several bat strains of coronavirus 229E, a virus previously detected in bats and related to the human strains
- Identification of rodent and bat adenoviruses thus shedding light into the diversity, evolution, and transmission of members of this virus family
- Detection of herpesviruses in primates and rodents, expanding our knowledge about herpesvirus hosts and distribution.

One particularly important feature of the PREDICT project in Cameroon is that we worked closely with ministerial partners at every phase of surveillance, from sample collection to laboratory analysis. The in-country PREDICT lab partner was the Cameroonian Military Health Research Center (CRESAR), the military health research lab that hosted the PREDICT team and supported the detection and identification of viruses.

VIRUS DETECTION

The PREDICT project was designed to not only detect those viruses which are known to cause human disease, but also related viruses that have yet to be discovered. By using specially designed primers during consensus PCR assays, the team was able to detect both known and new viruses. During PREDICT-1 (2009-2014), 20 virus groups were included in testing. In PREDICT-2 (2014-2019), we prioritized virus groups to focus on coronaviruses, filoviruses, paramyxoviruses, flaviviruses, and influenza viruses, results of which are reported in the virus table below.

There has been a tremendous amount of work done through the PREDICT project in Cameroon, including 4,220 animals sampled, 651 humans sampled and interviewed, and over 56,000 tests performed in the last five years. Through PREDICT-1 and PREDICT-2, 143 viruses were detected, and an enormous amount of associated metadata was collected. By carefully combing through and analyzing this data, the team made a number of highly impactful discoveries, to be shared with the public.

VIRUS FINDINGS IN PEOPLE

While dengue fever is a well-documented and highly studied disease in places like Southeast Asia, it is relatively overlooked in many parts of Africa, including Cameroon, where little is known about the prevalence of the disease. Our work led to the detection of the virus in two individuals from the South Region of the country who were infected with dengue virus (serotype 1), and who were also co-infected with malaria. Because dengue fever was not identified by the local hospital from which these people were enrolled, this finding indicates that dengue fever may be an underdiagnosed condition in Cameroon and supports the need to increase surveillance and treatment options for this flavivirus-caused condition in the area.

Additionally, hospital syndromic surveillance detected eight individuals with influenza A, two with paramyxoviruses, and one with a coronavirus. The paramyxoviruses were previously known viruses belonging to the parainfluenzavirus 2 and parainfluenzavirus 3 lineages, which are both relatively common causes of lower respiratory tract infections in humans. Interestingly, the parainfluenzavirus 3 is genetically very similar to a virus that other studies have also found in non-human primates. This positive individual was an infant enrolled from a hospital, whose parents reported that the child had no contact with non-human primates.

VIRUS FINDINGS IN WILDLIFE

During PREDICT-1, the project's work in Cameroon significantly increased the knowledge on the diversity of adenoviruses in bats in the region and has suggested new ways of understanding how these viruses evolve through factors in both the host and the viruses themselves, which may also be relevant to other viruses outside the adenovirus group. We also detected an adenovirus discovered in a moustached monkey (*Cercopithecus cephus*) that was more related to human adenoviruses than to other non-human primate varieties, perhaps suggesting the potential for increased risk of human infection by viruses from this primate lineage. Surveillance in rodents that were captured from nearby human settlements also found 14 new species of adenoviruses that were previously unknown to science.

VIRUS TABLE (2015-2019)

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Hospital (Dja et Lobo Division)	1	0	1
	PREDICT_CoV-30	Woermann's Fruit Bat	Ebolowa, Sangmelima	2	2	0
	PREDICT_CoV-35	Franquet's Epauletted Fruit Bat, Nut-Colored Yellow Bat	Nybissan, Sangmelima	2	2	0
	PREDICT_CoV-44	Noack's Roundleaf Bat	Sangmelima	11	5	6
	PREDICT_CoV-54	Giant Roundleaf Bat, Commerson's Roundleaf Bat	Sangmelima	15	5	10
	PREDICT_CoV-66	Little Collared Fruit Bat	Ebolowa	1	1	0
	PREDICT_CoV-70	Halcyon Horseshoe Bat	Sangmelima	1	1	0
	PREDICT_CoV-81	Noack's Roundleaf Bat, Sooty Roundleaf Bat	Sangmelima	19	15	4
	PREDICT_CoV-97	Egyptian Fruit Bat	Sangmelima	1	1	0
	PREDICT_CoV-109	Halcyon Horseshoe Bat	Sangmelima	1	1	0
	Bat coronavirus Hipposideros	Noack's Roundleaf Bat	Sangmelima	2	1	1
	Bat coronavirus HKU9	Egyptian Fruit Bat	Sangmelima	4	4	0
	Coronavirus 229E (Bat strain)	Noack's Roundleaf Bat, Short-Tailed Roundleaf Bat, Unidentified Hipposideros Bat	Ebolowa, Sangmelima	39	33	6
	Eidolon bat coronavirus	Straw-Coloured Fruit Bat	Maroua	1	1	0
	Kenya bat coronavirus/ BtKY83/59/58	Halcyon Horseshoe Bat	Sangmelima	1	1	0
	Zaria bat coronavirus	Giant Roundleaf Bat	Sangmelima	7	5	2
	PREDICT_CoV-98	Goliath Shrew	Sangmelima	1	1	0
	PREDICT_CoV-75	African Palm Civet	Ebolowa	1	1	0
Paramyxovirus	Human parainfluenzavirus 2	Human	Hospital (Dja et Lobo Division)	1	0	1
	Human parainfluenzavirus 3	Human	Hospital (Dja et Lobo Division)	1	0	1
	PREDICT_PMV-79	Noack's Roundleaf Bat, Unidentified Hipposideros Bat	Ebolowa	2	2	0
	PREDICT_PMV-80	Giant Roundleaf Bat	Town in Dja et Lobo Division, Sangmelima	2	1	1
	PREDICT_PMV-82	Giant Roundleaf Bat	Town in Dja et Lobo Division	1	1	0
	PREDICT_PMV-97	Noack's Roundleaf Bat	Ebolowa	1	1	0
	PREDICT_PMV-127	Noack's Roundleaf Bat	Sangmelima	1	0	1
	PREDICT_PMV-133	Noack's Roundleaf Bat	Sangmelima	1	1	0
	PREDICT_PMV-138	Noack's Roundleaf Bat	Sangmelima	2	2	0
	PREDICT_PMV-139	Short-Tailed Roundleaf Bat	Sangmelima	2	2	0
	PREDICT_PMV-147	Noack's Roundleaf Bat	Sangmelima	1	1	0
	PREDICT_PMV-152	Noack's Roundleaf Bat	Sangmelima	1	1	0
	PREDICT_PMV-161	Noack's Roundleaf Bat	Sangmelima	1	1	0
	PREDICT_PMV-163	Noack's Roundleaf Bat	Sangmelima	1	1	0
	PREDICT_PMV-91	Unidentified Crocidura Shrew	Sangmelima	1	1	0
	PREDICT_PMV-101	Unidentified Crocidura Shrew	Town in Dja et Lobo Division, Sangmelima	3	1	2
	PREDICT_PMV-131	Jackson's Soft-Furred Mouse	Town in Dja et Lobo Division	1	1	0
	PREDICT_PMV-148	Fire-Bellied Brush-Furred Rat, Goliath Shrew, Misonne's Soft-Furred Mouse	Town in Dja et Lobo Division, Sangmelima	3	3	0
	PREDICT_PMV-156	Goliath Shrew	Sangmelima	1	1	0
	PREDICT_PMV-160	Fire-Bellied Brush-Furred Rat	Town in Dja et Lobo Division	1	1	0
Influenza virus	Influenza A	Human	Hospital (Dja et Lobo Division), Sangmelima Hospital	5	2	3
	Influenza B	Human	Hospital (Dja et Lobo Division)	3	3	0
Flavivirus	Dengue virus serotype 1	Human	Hospital (Dja et Lobo Division), Sangmelima Hospital	2	2	0
Poxvirus	Monkey pox	Chimpanzee, Environmental Sample	Mefou	4	4	0
Total				**152**	**113**	**39**

IN-DEPTH ETHNOGRAPHIC INTERVIEWS & FOCUS GROUPS REVEALED:

- Some market workers and hunters interviewed said that bushmeat doesn't transmit illness to people, and that transmission of disease cannot occur between animals and humans.
- Some market workers and butchers say that working with wild animals is not risky. Many believe that the only risk is of cutting oneself, not due to blood-blood contact between animals and humans, but because the wound may get infected if not treated properly.
- Most vendors perform wound care when they cut themselves. Some report stopping bleeding by putting salt or lemon on the wound and others use alcohol and bandages.
- Most market workers and hunters do not consider PPE important. Several mention that gloves are not a feasible protective measure, as "hospital-style gloves are too thin to protect against anything, and larger gloves used for heavier tasks are too cumbersome for the work we do."
- According to a restaurant worker, people in Sangmelima do not hunt or consume bats, as their physical appearance is off-putting to many, with a few individuals explaining that they are too ugly to eat.
- Participants noted they received health advice from the Ministry of Health via telecom companies sending SMS text messages. Those interviewed see SMS texts as a positive intervention, with helpful messages and good health advice. This medium for behavioral change communication would be an effective strategy to continue and expand in Cameroon.

EPIDEMIOLOGICAL & BEHAVIORAL RISK

The PREDICT team conducted behavioral risk characterization in three sites across southern Cameroon, Dja et Lobo Division. Through this work, the team identified risk behaviors that may be associated with zoonotic disease transmission and communicated findings to participating communities to improve awareness of potential disease threats and opportunities for prevention and control.

The team regularly conducted community-based risk communication sessions with groups in frequent contact with wild animals – specifically bushmeat hunters, transporters, and sellers – and therefore were at higher risk for zoonotic disease. These sessions included discussions on zoonotic disease infection risks and potential risk mitigation strategies. The PREDICT team advised the bushmeat trade community to avoid handling or butchering fresh meat if their hands are cut or scratched, to always have soap and water nearby to wash immediately in case they are cut during butchering, to avoid contact with wildlife bodily fluids (using impermeable plastic to wrap meat during transport), to avoid contact with dead animals found in the forest, and to keep wildlife carcasses or bushmeat out of reach of children.

BEHAVIORAL TRENDS AND LONGITUDINAL ANALYSIS OF BUSHMEAT MARKET SPECIES & PRICES

Bushmeat markets are an area of concern when considering the possibility of zoonotic viruses making the leap from wild animals into humans. Understanding which animals are associated with zoonotic disease spillover and spread, which species are present at local markets, and the economic factors that perpetuate their sales, is critical to alleviating this risk. At two bushmeat market sites, the team recorded sales of nearly 40 different species of wild animals, many of which are protected species that are illegal to hunt or to sell, and several of which are endangered due to their low remaining population sizes in the wild and frequent involvement in illegal trafficking. Prices ranged widely for different species, and the demand for the wild meat was high.

Through interviews and questionnaires, we learned that peoples' involvement in the bushmeat value chain was much more intense in some areas of Cameroon than others, and that the hunting and butchering of certain species of animals – like non-human primates and rodents – is much more common than others, such as bats. We also found that men and older individuals are up to seven times more likely to hunt for these animals than young women, and also that older individuals considered these activities to be riskier than young hunters did. This information helps to target risk communication discussions to those groups where impacts will be highest. By engaging vendors and community members in group discussions about the risk of contracting diseases from wild animals, and ways to keep themselves healthy, the team is encouraging safe behaviors and alternatives to bushmeat to lower the overall risk of disease emergence.

These sessions also included behavioral change intervention sessions whereby the team conducted a guided activity designed to help people understand the risk of interaction with bats, reviewing images in the *Living Safely with Bats* book, a resource developed by the PREDICT project. The community asked questions and the team made recommendations for living more safely with bats and other animals that they interact with regularly.

STRENGTHENING CAPACITY

Following the 2017 WHO Joint External Evaluation, which found that Cameroon was lacking in wildlife disease surveillance capacity, the PREDICT team and the Food and Agriculture Organization (FAO) took action to bolster this capacity by jointly conducting three training sessions for 75 staff from the Ministries of Wildlife and Livestock. Further, the One Health Focal Point from the Ministry of Forests and Wildlife joined the PREDICT team to train 20 game rangers from protected areas around the country.

The PREDICT project helped to strengthen the workforce by training 85 veterinary students on zoonotic disease surveillance at two national veterinary schools. To preserve the initiatives created by USAID in Cameroon over the past 10 years, the PREDICT project participated in curriculum development for a Master of Science degree in Wildlife Health, with intake to begin in 2020 at the University of Buea. This program will be the product of a collaboration between OHCEA-Cameroon, Cameroon's Ministries of Higher Education (MINESUP), Forests & Wildlife (MINFOF), Livestock, Fisheries & Animal Industries (MINEPIA), University of Minnesota, Tufts University, FAO-ECTAD, USAID Preparedness & Response, and the PREDICT project in Cameroon.

OUTBREAK PREPAREDNESS & RESPONSE

The PREDICT project strengthened Cameroon's ability to investigate zoonotic disease outbreaks by providing on-the-job training to a vast number of people who engage in outbreak response activities. Government technical staff from the Ministry of Livestock, Fisheries and Animal Industries and the Ministry of Forestry and Wildlife who received training and hands-on experience with the PREDICT team in animal capture, safe handling, and sample collection techniques were on the front lines during a monkeypox outbreak in chimpanzees at Mfou Sanctuary in August 2016. The PREDICT team also provided support to the Military Health Research Center (CRESAR) and the National Veterinary Laboratory (LANAVET) to undertake diagnostic assays using PREDICT testing protocols to assist in the rapid identification of the monkeypox virus. We also assisted in the investigation of various wildlife die-off events in bats and gorillas, including confirmation of an influenza outbreak where H5N1 influenza A was identified at a poultry farm in Yaounde in 2016, followed by donating 100 pairs of disposable coveralls and 1,000 N95 masks to the Ministry of Livestock, Fisheries & Animal Industries for the response.

PRACTICAL IMPLICATIONS

The PREDICT project's work in Cameroon has been pivotal in moving the One Health platform from theory to practice. The project brought into focus the importance of zoonotic disease spillover in the Congo Basin, one of the hotspot regions of infectious disease emergence. With its emphasis on increasing capacity expansion, the project has reinforced human and animal health preparedness and response capabilities in the country, and have led One Health trainings for professionals across Cameroon and the region.

The project provided technical trainings for dozens of government workers, health and medical staff, veterinary and wildlife workers, and to students who will become the next leaders in global health. In addition to the core PREDICT project activities of continued disease surveillance in both animals and humans, the Cameroon team has, for nearly a decade, also performed vital services such as assisting with disease outbreak investigations, created opportunities for learning and awareness about zoonoses in bushmeat markets and with wildlife hunters, and have also spread the knowledge gained by this program across the world through both peer reviewed publications and by presentations at international conferences.

Over the past 10 years, the project has had important impacts in Cameroon, in terms of virus discovery, establishing stronger partnerships across Ministries, and through in-depth research in markets and hunting communities on behavioral risks to which communities are exposed in the light of the animal interfaces with which they regularly interact. In its efforts to promote strong and integrated global health security, the PREDICT project has made a clear impact and has supported Cameroon in advancing its capabilities in zoonotic emerging infectious disease detection and surveillance. The seeds have been planted and Cameroon will continue to grow from the strong roots that USAID has helped nurture through the PREDICT project.

REFERENCE

- Daszak, Peter, Andrew A. Cunningham, and Alex D. Hyatt. "Anthropogenic environmental change and the emergence of infectious diseases in wildlife." *Acta Tropica* 78.2 (2001): 103-116. DOI: 10.1016/S0001-706X(00)00179-0

SPECIAL FEATURE

BATS & PEOPLE: WHAT'S THE RISK IN CAMEROON?

To gain a more detailed understanding of the ways in which people in Cameroon interact with bats, which are known to carry many zoonotic pathogens, the PREDICT team traveled to distant locations to visit caves and forests where locals and tourists can be found mingling with bat populations.

Read more about this study at **bit.ly/2wdmLed**

For more information view the interactive report at **p2.predict.global**

PREDICT
CÔTE D'IVOIRE

ONE HEALTH IN ACTION (2014-2020)

Empowering One Health teams to strengthen national health security and preparedness for pandemic threats

Since 2016, the PREDICT project worked to build national capacity and raise awareness of the importance of One Health surveillance for prevention, detection, and response to zoonotic disease threats. The PREDICT Côte d'Ivoire team, locally based at Institut Pasteur du Côte d'Ivoire (IPCI) and the Laboratoire National d'Appui au Développement Agricole (LANADA), are key members of the technical working group on animal health. The PREDICT project provided a platform for partners to put One Health in action at the edges of the Marahoué National Park – an area that has been severely degraded by illegal agricultural activity and animal husbandry, increasing wildlife-human interaction. The team's surveillance and behavioral risk activities were focused in a 10 km radius around a local village within the Bouaflé Department, a community at-risk of zoonotic disease spillover and spread. Through the project, >350 people across animal and human health sectors–from the national to subnational levels–received hands-on training in zoonotic disease surveillance and disease detection techniques, and gained valuable experience with multisectoral collaboration and in implementing the One Health concept.

In addition, the PREDICT Côte d'Ivoire team worked to operationalize One Health in-country, organizing a practical workshop on wildlife monitoring at the Abidjan Zoo, and a training workshop on laboratory techniques for national laboratory staff in the human and animal health sectors. The team, along with the partners at the USAID PREPAREDNESS and RESPONSE project, contributed expertise to additional trainings and workshops that developed and validated operating documents using the Côte d'Ivoire's One Health platform. Collaboration between the PREDICT project and human, animal and environmental health stakeholders in Côte d'Ivoire helped establish a dialogue with these groups, resulting in the signing of a One Health decree by the ministries.

LOCAL PARTNERS

- Institut Pasteur du Côte d'Ivoire (IPCI)
- Laboratoire National d'Appui au Développement Agricole (LANADA)
- Abidjan Zoo; Ministry of Environment, Water and Forestry (MINEF)

>350

DEVELOPED the One Health Workforce by training more than 350 people in Côte d'Ivoire.

>1.1K

OPERATIONALIZED One Health surveillance and sampled over 1.1K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Institut Pasteur du Côte d'Ivoire (IPCI)
- Laboratoire National d'Appui au Développement Agricole (LANADA)

14,030
TESTS

3
KNOWN

DETECTED 3 unique viruses in both animal and human populations.

MIREILLE DOSSO

Director, *Institut Pasteur du Côte d'Ivoire*

"The PREDICT project has made it possible to create a multidisciplinary One Health group at the national level, and to better operationalize the concept in Côte d'Ivoire. The project has helped everyone to understand that the boundary between human and animal health is not a big one, and that a degradation in the environmental health, including deforestation, can have a significant impact on other health sectors."

ANTOINETTE KOUASSI LOU

Senior Health Laboratory Technician, *Laboratoire National d'Appui au Développement Agricole*

"My participation in PREDICT was an exceptional opportunity to work in another laboratory, to work in a team and especially, to work in a One Health setting with my colleagues at IPCI. I acquired a greater mastery of nested-PCR and improved my performance, given the large number of samples that had to be processed in a limited amount of time. This will allow me to have endurance and speed in the event of an epidemic or epizootic requiring a rapid and continuous diagnosis and response."

ROMEO GUEU KPON

Junior Laboratory Technician, *Institut Pasteur du Côte d'Ivoire*

"On a technical level, I was able to strengthen my skills in molecular biology, particularly the nested-PCR technique, troubleshoot problems especially those associated with laboratory contamination. I also learned to barcode animal species,and to clean and analyze the produced DNA sequences with opensource software. This has allowed me to further enrich my knowledge in the field of research."

ACHIEVEMENTS

- Provided a proof of concept of the value of One Health for emerging diseases from wildlife, fostering discussion of nationwide wildlife disease surveillance programs
- Built capacity for molecular identification of animal species, which can now be utilized to identify wildlife species seized in the context of wildlife trafficking
- Detected a known influenza A virus in people and two known coronaviruses in three bat species
- Collectively engaged stakeholders involved in human, animal, and environmental research, which led to the signing of the One Health platform decree
- Facilitated the creation of the Technical Working Group on Animal Health (GTTSA), a significant achievement in cross-sectoral collaboration and zoonotic disease surveillance
- Together with the PREDICT Liberia team and FAO, held a joint training to share experiences in the fight against epidemics and train Ivorian officers in wildlife monitoring, contributing to strengthened linkages between the teams and opening opportunities for collaboration and support in case of an outbreak in the border area
- Held a training workshop on the "Operationalization of One Health in Côte d'Ivoire" in collaboration with FAO and the One Health platform, which improved collaboration among multiple sectors, and made strides towards greater integration of the environment, rural development, finance, and security sectors
- Empowered local communities with knowledge and awareness of zoonotic diseases and worked with community members on risk reduction and behavior change communication strategies aimed at living safely with wildlife and balancing health and conservation goals

ONE HEALTH SURVEILLANCE

In Côte d'Ivoire, the PREDICT project carried out One Health surveillance activities at the edges of the Marahoué National Park between Bonon and Bouaflé, safely sampling and testing 293 rodents, 483 bats, and 401 humans. Established in 1968, the park occupies an area of approximately 100,000 hectares (ha) but was greatly impacted by an invasion of illegal cocoa planters and poachers. The subsequent intensification of agricultural activities and animal husbandry led to increased wildlife-human interactions. PREDICT project zoonotic disease surveillance activities were focused in a local village within the Bouaflé Department, which consists of three subsites within a 10km radius from the village center. The Bonon and Bouaflé clinics were chosen for human syndromic surveillance, since these clinics comprise the catchment area for the rural villagers included in our community-based surveillance efforts.

VIRUS DETECTION

In Côte d'Ivoire, virus screening of 2,554 specimens including oral, nasal, rectal swabs, and fecal samples from wildlife and humans was carried out predominately by IPCI, as well as LANADA. Both laboratories used standardized PREDICT protocols to screen for both priority zoonotic viruses of national public health concern, along with other novel and emerging viral threats. Wildlife and human specimens were tested using consensus PCR (cPCR) to screen for priority zoonotic viral diseases and emerging threats such as filoviruses (e.g. Ebola and Marburg), influenza viruses, coronaviruses, paramyxoviruses, and flaviviruses. Virus findings were confirmed through genome sequencing and interpreted to better understand the relationship of the detected sequence to those from known animal and human pathogens.

SPECIES IDENTIFICATION

Animal species were morphologically identified in the field and confirmed by molecular techniques (genetic barcoding) at IPCI, a new method for this lab made possible through the PREDICT project that is also enabling accurate species identification of animals seized in wildlife trafficking.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS
Coronavirus	Chaerephon bat coronavirus/Kenya/2006	Little Free-Tailed Bat, Unidentified Molossid Bat	Rural village (Bouaflé area)	2
	Kenya bat coronavirus/ BtKY56/BtKY55	Buettikofer's Epauletted Fruit Bat	Rural village (Bouaflé area)	1
Influenza virus	Influenza A	Human	CSU Bonon Clinic	1
Total				**4**

VIRUS FINDINGS IN PEOPLE

A known influenza A virus was detected in one adult female (out of 401 participants) sampled and tested from the Bonon clinic. Further investigation is required to determine the virus subtype. Influenza viruses are important pathogens in humans and animals. Virus discoveries will help hospitals in the Marahoué National Park region in their diagnosis when classifying any feverish patient with respiratory symptoms. No individuals from community-based sampling efforts were positive for any targeted viruses.

VIRUS FINDINGS IN WILDLIFE

Two known viruses were detected in three individual bats (of 483 sampled and tested) living in and around homes and communities in the Marahoué National Park region. The known Alphacoronavirus strain (Chaerephon coronavirus/Kenya/KY22/2006) was detected in fecal samples from two free-tailed bat species, an unidentified *Mops* sp. and the little free-tailed bat, *Chaerephon pumilus*, both of which are insectivorous and found in human dwellings in the region. In addition, a known Betacoronavirus strain (Kenya bat coronavirus BtKY56/BtKY55) was found in Buettikofer's epauletted bat, *Epomops buettikoferi*, a frugivorous species observed feeding on mango trees in the community. The genus *Betacoronavirus* includes viruses that are important to public health such as SARS and MERS, but this particular virus is not considered to be closely related to either of these viruses. Therefore, at this time, there is no evidence that these two strains pose a threat to human health.

All virus findings are contributing to: 1) risk analysis and characterization efforts to inform risk communication strategies and community awareness campaigns (leaflets, pooled results, posters explaining the risks); and 2) assessments of rodent and bat diversity and their role as disease carriers in disturbed habitats of the Marahoué National Park (due to land use change).

***Chaerephon pumilus** in a house of a patient sampled in PREDICT/Côte d'Ivoire concurrent site during field surveys in August 2018.*

PHOTOS: Eugène Kouassi Koffi

EPIDEMIOLOGICAL & BEHAVIORAL RISK

SYNDROMIC SURVEILLANCE

Since the beginning of the PREDICT project in Côte d'Ivoire, our team worked in the Bouaflé clinic and slaughterhouse, the CSU Bonon clinic, and several rural sites in the area, in addition to working with wildlife in the southwestern region at a rural site in Taï forest. Syndromic surveillance activities mainly focused on Bonon and Bouaflé areas. At the clinics, the PREDICT team enrolled 435 patients whom completed a questionnaire, 401 of which also consented to provide biological samples. Enrolled individuals were mostly women and adults residing in the communities around the main village, and in the catchment area of the Bonon and Bouaflé clinics. The most common symptoms of enrolled patients were fever with headache and severe fatigue or weakness, fever with muscle aches, cough, cold, sore throat, and fever with diarrhea or vomiting. Such illnesses were detected among all age groups, activities, and sites, but more often in individuals from rural than urban communities.

INSIGHTS FROM THE HUMAN QUESTIONNAIRE

Hunting and livestock breeding were carried out exclusively by men in both villages and urban areas. Men were also more involved in agricultural activities than women, who reportedly spent most of their time at home carrying out domestic tasks and activities not involving live animals (food preparation, cooking, trade). On the other hand, in more urban sites like the cities of Bonon and Bouaflé, women dominated the value chain related to animal business, namely game trade and wild animal restaurants. These findings suggest that wildlife-borne zoonotic agents might be introduced in family clusters by men at higher risk of contact with infected wild animals due to their hunting and farming activities and then spread via the game and meat trade in towns (including domestic and wild meat fresh products).

A total of 423 people in apparently good health were also surveyed in the rural community and surrounding camps. Individuals surveyed practiced farming and livestock breeding as main livelihood activities, with hunting reported as an alternative means of providing meat for their families; a few also reported being professional hunters or wild meat traders.

INSIGHTS FROM IN-DEPTH BEHAVIORAL RISK INVESTIGATIONS

The local behavioral risk team also led investigations into the human drivers of zoonotic disease transmission across seven sites in Côte d'Ivoire. These in-depth, one-on-one interviews and focus group discussions gave local social scientists the opportunity to probe the different contexts in which people of varying backgrounds interacted with live animals and bushmeat. As a taxa of particular interest, participants were asked to discuss the different ways in which they came into contact with bats at home and during their work days.

Among those who described hunting and slaughtering practices, the use of a slingshot, a catapult, or a gun/rifle were among the most cited ways to catch bats. Participants detailed how this method often necessitated killing the bat with a final blow of a machete once it fell onto the ground. Of note, some participants described specifically handing off the dead bats to children for preparation. When handling the dead bat, respondents described bare-handed contact with the body and with the bat blood. Several respondents detailed both current and previous bat consumption, many times regarding the taxa as appetizing. Some respondents also revealed that according to tradition, bat consumption was discouraged among pregnant women.

Possibly due to previous community messaging regarding Ebola transmission risk, respondents seemed uneasy discussing bats with the interviewers. Eating the animal was described by some as a custom of the past, with others limiting their discussion to a simple expression of distaste for the taxa. However, while some respondents reported bats as currently being rare or difficult to find, others described their regular presence around their homes and in their fields. These apprehensive or negative sentiments towards bats might possibly be linked to institutional and historical distrust from previous Western interventions, imprecise public health messaging, and a 'seeing is believing' understanding of Ebola.

> “Well, it was in the field, in a big tree. Someone had a slingshot and he killed them...once it is killed, we take it to the field and burn it a little and skin it, we wash it and then the women cook it...we do it with our bare hands and we touch the blood.”

> “Well, there's a tree called goyafa [guava]. Bats like to eat that. So, when it's evening, I go and sit underneath it and then I start shooting at them with a slingshot. I can kill 5 or even 6.”
>
> –Adult male crop

The insights gained through the in-depth behavioral and social science work provided invaluable information for the development of culturally appropriate, livelihood sparing interventions and behavior change communication campaigns designed to reduce risk of viral spillover from wildlife hunting and bushmeat consumption while also promoting wildlife conservation and the critical ecosystem service roles bats play. Researchers and health workers need to remain mindful of previous education and awareness campaigns as brought up by the interview respondents, and the impacts lasting impressions, knowledge, and possible misunderstandings local residents have of well-intentioned messaging.

> "Yes, we heard about something like that, it is said that bats give Ebola and monkeys transmit AIDS, but I do not know if it is true...We heard about it. We were scared, we watched out, but then they said Ebola was over."

> "There are two kinds [of bats].Those which are in the houses, we do not eat them...the little ones which are in the houses are the ones with a lot of diseases."

–Focus group of adult male hunters,
trappers and fishers

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

The PREDICT team engaged in risk communication and result dissemination at the community level to foster improved awareness of zoonotic diseases and transmission pathways, along with potential prevention and control strategies. At each field site visit, our team communicated the potential health dangers that wildlife, particularly bats, could represent. These discussions were beneficial to both the communities and the PREDICT team who learned from the villagers' perceptions of risk, and together worked to identify solutions to their concerns.

The villagers were encouraged to preserve the bats' roosting sites, but to avoid bats taking refuge in their houses and becoming a direct threat. Due to the proximity of bats to humans in some villages, the PREDICT team conducted community outreach using the *Living Safely with Bats* behavior change and risk communication resource published by the PREDICT Consortium. Copies of the bat book were distributed to the representatives of the communities during the visit in April 2019, and a presentation of *Living Safely with Bats* was also conducted within the village via projector. In addition, during the joint FAO, IPCI, LANADA, DSV and PREDICT training mission, farmers were made aware of the disadvantages of wandering livestock farming, the advantages of vaccinating animals, and the economic importance of rural livestock.

CAPACITY STRENGTHENING

In Côte d'Ivoire, the PREDICT project fostered discussion about the need for, and developed capacity for a nationwide One Health wildlife disease surveillance program. Before the PREDICT project, only isolated initiatives were dedicated to specific wildlife diseases, usually in the context of research programs.

LABORATORY SYSTEMS STRENGHTENING

The PREDICT project, in collaboration with Columbia University, organized training sessions to increase capacity within national laboratories for the detection of priority virus groups. Key workers were trained in PREDICT project protocols, laboratory techniques, biosecurity and biosafety, sample storage and labeling, and database management. In addition, the PREDICT project was directly involved in the Ivorian laboratory system's capacity building through provision of equipment, reagents, and consumables, selection for optimal virus detection, and professional development of the laboratory staff. Portable freezers were provided to IPCI to ensure cold chain; allowing for the optimal conservation of viral material during an outbreak response. USAID, through FAO, also provided similar equipment to LANADA, allowing the country to possess strong cold chain capacity to work in remote areas, ensure samples were stored correctly and enable zoonotic disease surveillance in the field.

JOINT WILDLIFE SURVEILLANCE TRAINING WITH THE PREDICT LIBERIA TEAM, IPCI, & FAO

In June 2019, 50 participants joined a workshop and field training, facilitated by PREDICT teams from Côte d'Ivoire and Liberia at the Institut Pasteur du Côte d'Ivoire and Abidjan Zoo. Participants from a range of stakeholders advanced their knowledge and skills for pathogen surveillance in wildlife, including attendees from headquarters and regional offices of the Director of Veterinary Services (DSV), the Director of Wildlife and Hunting Resources (DFRC), the Ivoirian Office of Parks and Reserves (OIPR), the National Agency for Rural Development Support (ANADER), the Directorate of Health and Social Action of the Armed Forces (DSASA), Vision Verte, the IPCI, and LANADA.

The PREDICT teams conducted seminars on occupational health hazards linked to snake bites and venom, zoonotic agents circulating in wildlife in the region, and anesthesia of large mammals. Participants also completed hands-on field training in the PREDICT project's safe and humane wildlife surveillance techniques, including rodents at IPCI, and bats and rodents at the Abidjan Zoo.

This workshop reinforced collaboration between cross-border teams and illustrated the possibility of working among sectors and agencies from all over the country to discuss issues and methods for improving sharing of epidemiological animal data.

OUTBREAK PREPAREDNESS & RESPONSE

DOMESTIC ANIMAL DISEASE OUTBREAK PREPAREDNESS

Due to successful trainings, the PREDICT project's functional virus testing platform, and the necessity for harmonized outbreak preparedness, FAO funded a training at our One Health surveillance site near Marahoué National Park to sample domestic animals with technical assistance provided by the PREDICT team. All participants were trained in PREDICT project biosafety and humane animal capture and sampling protocols, complied with occupational health best practices including required vaccinations, and operated under the direction of the Veterinary Services authority. During this training, several preventive veterinary interventions were implemented as domestic carnivores were vaccinated against rabies, poultry were vaccinated against Newcastle disease virus, and ruminants were given an anti-helminthic.

CROSS BORDER OUTBREAK PREPAREDNESS

PREDICT Côte d'Ivoire and PREDICT Liberia teams, with the support of FAO, organized a joint training to share knowledge and experiences in the fight against epidemics and to train Ivorian officers in wildlife monitoring. This unique training contributed to establishing strong links between the teams, and to harmonizing sampling techniques to facilitate working jointly in case of an outbreak in the border area, or in the interior of Côte d'Ivoire.

PRACTICAL IMPLICATIONS

- In Côte d'Ivoire, the PREDICT team contributed to the design of national global health indicators, and also worked to include wildlife surveillance in the national health safety plan.
- The PREDICT project brought together stakeholders involved in human, animal, and environmental health to research and reach consensus and sign the One Health platform decree in Côte d'Ivoire.
- The PREDICT project and partners facilitated the creation of the Technical Working Group on Animal Health (GTTSA). GTTSA is a significant achievement in cross-sectoral collaboration and zoonotic disease surveillance in Côte d'Ivoire.
- Rich insights gained from the in-depth behavioral work are invaluable for designing culturally appropriate messaging and community engagement campaigns on wildlife conservation and spillover risk reduction.
- To sustain our community outreach and risk communication efforts, the National Rural Development Support Agency (ANADER), the government entity that works closely with rural communities on agricultural and livestock issues, continues to use the *Living Safely with Bats* resource as a tool to communicate with villagers about potential risks from bat-borne zoonoses and balancing health and conservation goals.
- The PREDICT project directly contributed to strengthening the national laboratory system's capacity for zoonotic disease detection through provision of equipment, reagents and consumables, and training for laboratory staff from both the IPCI and LANADA, Bingerville laboratories.

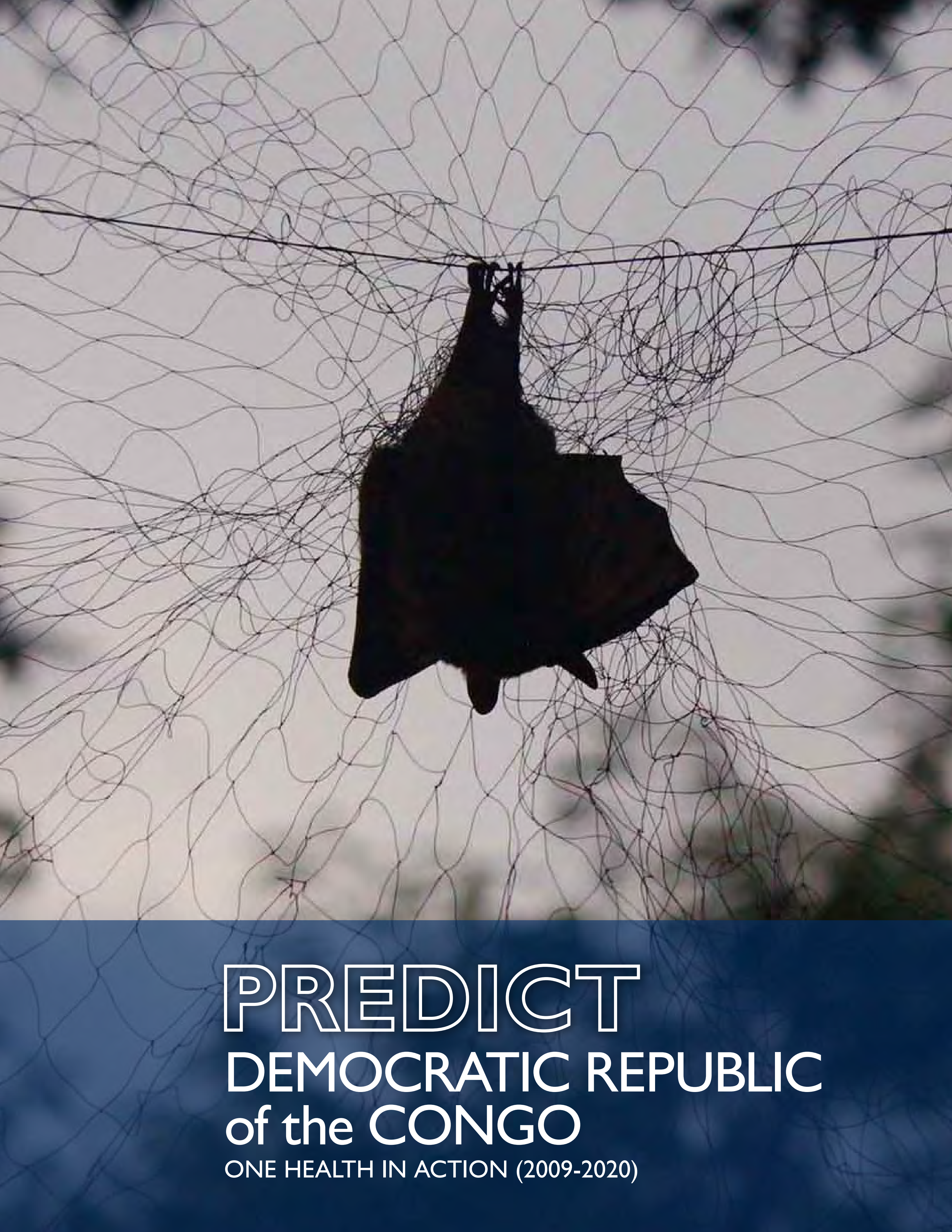
SPECIAL FEATURE

OPERATIONALIZING ONE HEALTH IN CÔTE D'IVOIRE

PREDICT organized a workshop at the Institut Pasteur du Côte d'Ivoire (IPCI) on operationalizing One Health in the country. Putting the World Bank One Health Operational Framework to use, the workshop focused on practical, value-added ways to integrate multi-sectoral collaboration, bringing together authorities from Ministries of Health, Veterinary Services, Wildlife, Environment, Security, Rural Development, and Economics and Finance as well as local NGOs and academic partners. An exercise on investing in One Health used three case studies to examine multi-sectoral impacts of African Swine Fever (ASF), lagoon pollution, and impacts from construction of the Soubre dam.

Read more at **bit.ly/2X7vCbx**

For more information view the interactive report at **p2.predict.global**



PREDICT
DEMOCRATIC REPUBLIC of the CONGO
ONE HEALTH IN ACTION (2009-2020)

DEMOCRATIC REPUBLIC OF THE CONGO (WEST)

Improving One Health surveillance capabilities to detect (re)emerging disease threats in the heart of the Congo Basin

WESTERN DR CONGO:

Throughout the central African region, demand for wild animal meat (also known as 'bushmeat') is constant, with as much as one million metric tons of bushmeat eaten each year in the Congo Basin. The Democratic Republic of the Congo (DRC) is home to endangered wildlife, including forest elephants, chimpanzees, bonobos, and lowland and mountain gorillas who inhabit the lush forests. Unfortunately, the biggest threat and leading cause of wildlife loss in the Congo Basin is the commercial bushmeat trade, driven by an ever-increasing market. As was heard during interviews with vendors by PREDICT project staff, many wealthy city dwellers indicate a preference for wild meat and will pay a premium for it, even when their protein requirement can be met with cheaper sources (Foerster et al., 2012; Schenck et al., 2006). Serving bushmeat from protected species is a status symbol in urban populations and harkens back to the nostalgia of hunting traditions in the village. Vigorous trading of wild meat to satisfy urban demand is widespread in all major Central African cities (Hennessey & Rogers, 2008; Mbete et al., 2011), including in the great metropolis of Kinshasa, DRC's capital city of nearly 12 million people.

For these reasons, our team adopted a specific focus on the bushmeat trade and movement of wild animal meat along the animal value chain from nearby hunting and trapping communities in Kongo Centrale, which serves as a source of wild animal meat feeding into the bushmeat markets in Kinshasa. In addition to targeting markets in the capital city, we conducted syndromic disease surveillance in two large hospitals in Kinshasa, which are proximate to key bushmeat markets, and where market workers go for affordable medical care when they are ill.

We initiated enhanced, coordinated surveillance capacity between members of the Ministry of Health, the Ministry of Fisheries and Livestock, and the Ministry of Environment to explore the high-risk disease transmission interfaces. Since 2009, the PREDICT project in Western DRC was implemented by the Institut National Recherche Biomédicale (INRB), DRC's premier reference lab, in close partnership with the Kinshasa School of Public Health, private partners, public hospitals, and local communities.

EASTERN DR CONGO:

From 2014-2019, the PREDICT project has focused zoonotic virus surveillance in wildlife and people in the North and South Kivu Provinces because the region presents complex challenges at the wildlife-human interface. Virunga National Park (PNVi) in North Kivu is a large and highly biodiverse protected area with abundant wildlife resources, but is a place where militia and rebel groups routinely set up camps inside the park, hunters and harvesters illegally enter the park to set snares and make charcoal, and community members utilize bat roosting caves inside and adjacent to the park as water sources. PNVi is also visited by international tourists who come to trek human-habituated mountain gorillas. Generally speaking, the area sees a tremendous flux of people crossing DRC's borders with Uganda and Rwanda. PREDICT worked inside the Mikeno sector of PNVi and in the adjacent communities, capturing and sampling primates and bats. As well, PREDICT enrolled consenting febrile patients (including park workers) presenting to health clinics in surveillance activities, collecting biological samples and administering questionnaires to better understand the extent to which patients were in contact with wildlife. PREDICT also worked in and around Kahuzi-Biega National Park in South Kivu Province, where militia and rebel groups are engaged in illegal activities inside the park (such as mining and bushmeat hunting), where subsistence agriculture occupies the corridor between the park's highlands and lowlands, and where a national road transecting the park brings heavy traffic of people and livestock into the park in vehicles and by foot from Tanzania, Uganda, Burundi and Rwanda. Adjacent to PNKB is the Centre de Réhabilitation des Primates de Lwiro, which receives and cares for confiscated and rescued wildlife, mainly great apes and other primates.

In North and South Kivu, the PREDICT team collected samples from 141 non-human primates, 289 bats, 12 rodents and 416 febrile human patients. Molecular methods (PCR) were used at the Institut National de Recherche Biomédicale (INRB) in Kinshasa and at the University of California, Davis One Health Laboratory to screen samples for evidence of infection with or exposure to potentially zoonotic pathogens of pandemic potential.

DEMOCRATIC REPUBLIC of the CONGO

LABORATORY STRENGTHENING

- Institut National de Recherche Biomédicale (INRB)

52,958
TESTS

22 PREDICT

29 KNOWN

DETECTED 51 unique viruses in both animal and human populations.

Global Health Security Agenda

DEMOCRATIC REPUBLIC OF THE CONGO (WEST)

>100

DEVELOPED the One Health Workforce by training more than 100 people in DR Congo.

>5.8K

OPERATIONALIZED One Health surveillance and sampled over 5.8K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LOCAL PARTNERS

- United States Centers for Disease Control (CDC)
- Central Veterinary Laboratory of Kinshasa
- Food and Agriculture Organization
- Institut Congolais pour la Conservation de la Nature (ICCN)
- Institut National de Recherche Biomédicale (INRB)
- Kinshasa School of Public Health (KSPH)
- Lola Ya Bonobo Sanctuary, Kinshasa
- Metabiota, Inc.
- Ministry of Environment
- Ministry of Fisheries and Livestock, Direction de la Production et Santé Animale (DPSA)
- Ministry of Health
- Mountain Gorilla Veterinary Project, Inc. (MGVP, Inc) aka Gorilla Doctors
- WHO DRC Disease Surveillance team

CHARLES KUMAKAMBA

Country Coordinator, *Metabiota*

"The PREDICT project has enabled young scientists like myself to improve our knowledge of microbiology and sample collection through its protocols. With PREDICT, I gained much experience and grew scientifically."

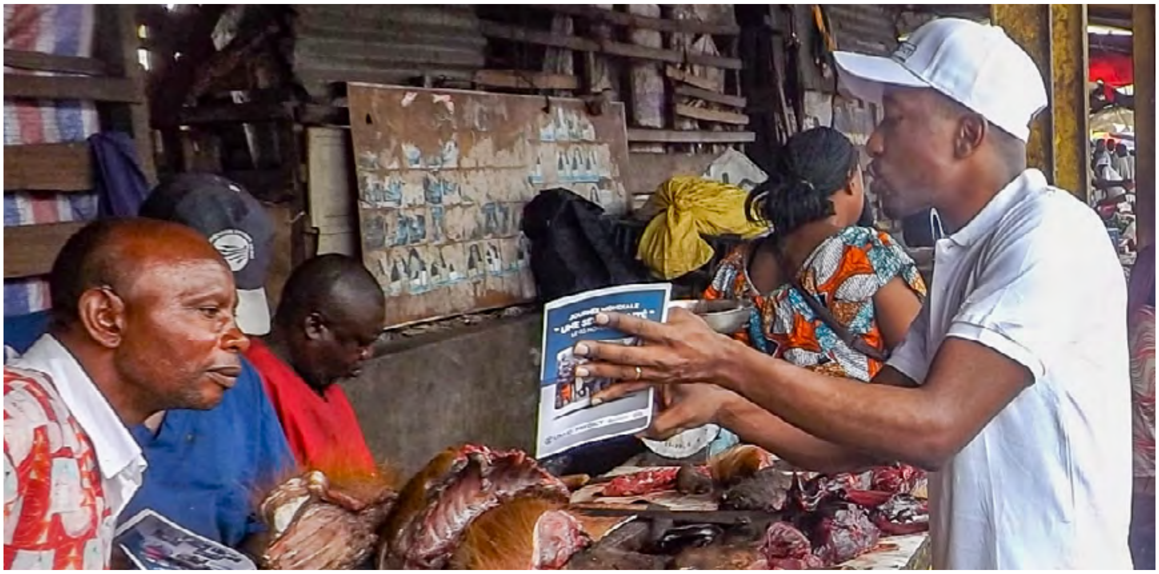
MARIA MAKUWA

Laboratory Advisor & Administrative Coordinator, *Metabiota*

"I have benefited from learning new methods of detection and characterization of viral pathogens as part of the PREDICT project. My training as a researcher in the laboratory has been enriched by field work and exchanges with the local populations – activities hitherto unknown to me. This program also allowed me to share my knowledge with my young colleagues and learn from their experience."

ACHIEVEMENTS

- Detected bocavirus DNA resembling human bocaviruses in 6 wild non-human primates, which indicates virus sharing; uncovered evidence of more than a dozen different bat coronaviruses, which are of high interest in light of past outbreaks of SARS and MERS; amplified 20 different herpes viruses from over 100 primates and rodents, shedding more light onto the diversity of this virus group; and identified 3 novel species of rodent adenovirus DNA, highlighting how little we know about the hosts of this virus family
- Confirmed the presence of *Zaire ebolavirus* RNA from a human, as part of an outbreak response where the PREDICT team assisted the government of DRC in identifying the causative agent of an unknown hemorrhagic fever and yielding the first sequence of this outbreak's strain, contributing to the rapid containment of the epidemic
- Organized an International One Health Day zoonotic disease awareness campaign at the La Liberté and Ndolo markets where bushmeat is sold, and held discussions with market vendors, managers, and patrons about the possibility of disease transfer between wild animals and humans while distributing information on how to keep safe from infection
- Helped establish functional communications and collaboration between animal and human health organizations enabling ministries to better communicate research findings, organization goals, and challenges, thus demonstrating how the One Health framework can provide value to national health security
- Conducted 179 semi-structured qualitative interviews and 12 focus groups with vendors, butchers, and other actors in the animal value chain and identified risky behaviors, interfaces, and key areas to inform continued and targeted surveillance, as well as risk reduction recommendations for improved health security



ONE HEALTH SURVEILLANCE

The PREDICT project's One Health surveillance approach was designed to strengthen capacity for detection of emerging viral threats and to improve our understanding of risk of zoonotic diseases in communities with close and frequent animal contact. Our team sampled wildlife, including both live animals and dead animals being sold for food (also known as "bushmeat"), and people concurrently, targeting high-risk populations for viral spillover, amplification, and spread in rural and highly urbanized settings in DRC. We also conducted syndromic surveillance of patients presenting to local hospitals serving the communities. Data collection and sampling was performed longitudinally over a three-year period during both the rainy and dry seasons, and identification of bat and rodent species was confirmed using DNA barcoding, a molecular-based laboratory assay.

The PREDICT team collected animal samples from nine bushmeat markets around Kinshasa. Through hospital based syndromic surveillance, the PREDICT Western DRC team enrolled 418 sick individuals at St. Joseph and Kingasani hospitals in Kinshasa targeting individuals with acute febrile illness with the intention of detecting zoonotic viruses among occupationally exposed humans. The team further explored the high-risk animal-human interface of bushmeat markets by conducting qualitative research with bushmeat vendors, butchers, transporters, and middlemen, to explore practices and behaviors with the potential to facilitate spillover and spread of zoonotic viruses.

The PREDICT team also worked with local communities to increase awareness on One Health and the benefits of the approach for living healthier lives. For example, in November 2018 the team organized an International One Health Day awareness campaign at the La Liberté and Ndolo markets in Kinshasa, where bushmeat is sold. The team had discussions with market vendors, managers, and patrons about the possibility of pathogen transmission between wild animals and humans and handed out outreach materials with suggestions on how to keep themselves safe around live animals and bushmeat.

Through coordinated animal and human surveillance, the PREDICT Western DRC team succeeded in improving coordination between animal and human health government sectors, national NGOs, and international organizations through regular consultation and data sharing, thus allowing for early awareness of public health alerts and animal die-offs. By bringing members of the Ministry of Health, the Ministry of Fisheries and Livestock, and the Ministry of Environment together to reach a common goal, the PREDICT project has demonstrated how the One Health framework can provide valuable benefits to all partners.

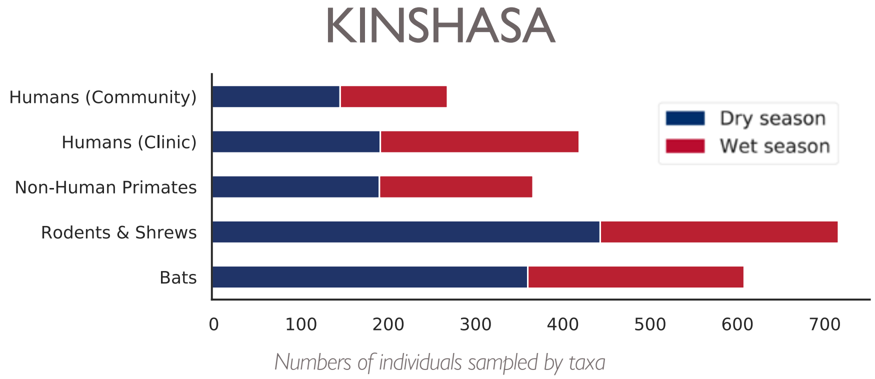
SURVEILLANCE SITES

KINSHASA

bats; humans; non-human primates; rodents/shrews

BUTA

bats; non-human primates; rodents/shrews



VIRUS DETECTION

The PREDICT project was designed to not only detect those viruses which are known to cause human disease, but also unknown viruses. This was done through the use of consensus PCR, a technique that uses specially-designed, broadly-reactive primers capable of amplifying genetic material of both known and novel viruses. During the first phase of the PREDICT project (PREDICT-1; 2009-2014), 25 different assays capable of detecting a diverse array of virus groups were included in surveillance activities. In PREDICT-2 (2014-2020), however, the number of virus groups investigated was reduced, in order to significantly increase the focus on those which are known to harbour the most dangerous human pathogens with pandemic potential, namely: coronaviruses, filoviruses, paramyxoviruses, flaviviruses, and influenza viruses.

Throughout the 10 years of PREDICT project work in DRC, several viruses were detected. Some of the highlights include the detection of bocavirus DNA resembling human bocaviruses in several wild non-human primates, suggesting cross-species transmission (Kumakamba et al., 2018); uncovering several different bat coronaviruses, which are of high interest in light of past outbreaks of SARS, MERS, and the novel 2019 coronavirus, SARS-CoV-2, originating from Wuhan, China; and the detection of many herpesviruses from over 100 primates and rodents, which is shedding more light onto the diversity of this virus group. PREDICT project testing also confirmed the presence of *Zaire ebolavirus* RNA from a human, which was part of an outbreak investigation in which the PREDICT project assisted the government of DRC in identifying the causative agent of an unknown hemorrhagic fever. This work yielded the first sequence of this outbreak's strain and helped contribute to the rapid containment of the epidemic. Importantly, it was also the first instance of a domestically confirmed case of Ebola virus disease (EVD) in DRC.

In PREDICT-2, the DRC team identified four unique coronaviruses in 21 individual bats belonging to both fruit-eating and insect-eating bat families. These coronaviruses are known viruses and are not believed to pose a threat to human health. We also detected one novel paramyxovirus, PREDICT_PMV-15, in three individual free-tailed bats (*Mops condylurus*) from Kinsasha. In addition to wildlife virus findings, we also detected influenza B in a 4-year old child who presented to the hospital in Kinsasha with acute febrile illness.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS
Filovirus	Ebola Virus (EBOV)	Human	Bikoro	1
Coronavirus	Bat coronavirus Hipposideros	Noack's Roundleaf Bat	Buta	1
	Chaerephon bat coronavirus/Kenya/KY22/2006	Angolan Free-Tailed Bat, Unidentified Chaerephon Bat	Kinshasa	9
	Eidolon bat coronavirus	Straw-Coloured Fruit Bat	Kinshasa	8
	Kenya bat coronavirus/BtKY56/BtKY55	Peter's Dwarf Epauletted Fruit Bat, Unidentified Myonycteris Fruit Bat	Kinshasa	3
Paramyxovirus	PREDICT_PMV-15	Angolan Free-Tailed Bat	Kinshasa	3
Influenza virus	Influenza B	Human	Kingasani Hospital (Kinshasa)	1
Total				**26**

EPIDEMIOLOGIC & BEHAVIORAL RISK

IN-DEPTH ETHNOGRAPHIC INTERVIEWS & FOCUS GROUPS

A major focus of PREDICT's behavioral surveillance in DRC was exploring the animal-human interface in the bushmeat markets in Kinshasa, elucidating important behavioral risk and zoonotic disease exposure dynamics. Throughout PREDICT-2, the DRC team conducted 179 semi-structured qualitative interviews and 12 focus groups with vendors, butchers, and other actors in the animal value chain. Interviews took place in Kinshasa bushmeat markets, in nearby hunting villages in Kongo-Central, in Mbandaka where much of the hunted non-human primates are sourced that feed into the Kinshasa markets, and in Buta, Bas-Uele province in northern DRC.

Vendors and others who work in the bushmeat trade were interviewed about behavioral risk, wild animal contact, and disease exposure. Most participants expressed a general lack of knowledge of disease transmission, particularly about the role of animals in the transmission of illness. Most vendors reported that wild animals cannot carry disease and therefore could not transmit diseases to humans. Bushmeat is perceived to be "natural" since it is not raised by humans. Some said they had heard Ebola was spread by animals but others attribute Ebola to witchcraft. Multiple bushmeat vendors stated, "This story of Ebola is false. There was a trapper whose animal was stolen and people ate it, and to get revenge he made a fetish and he killed all those who had eaten his meat....[clients] continued to eat bushmeat because they knew that it wasn't Ebola but rather a history of bushmeat and witchcraft."

HOSPITAL-BASED SURVEILLANCE & COMMUNITY WORK

Based on the questionnaires administered to hospital patients and individuals involved in the animal value chain, 52% of respondents reported consuming raw or undercooked meat, and 26% reported having slaughtered an animal in the past year. Nearly 75% of participants said they are worried about diseases in live animals in their local markets. However, participants showed a lack of understanding about the risks associated with slaughtering or butchering animals when they have an open wound, with over half responding "I don't know" when asked if there is any risk involved. The majority of individuals reported visiting a hospital or clinic when ill, while 7% reported visiting a traditional healer.

Of the people enrolled from both hospital and community settings, 39% had reported a fever within the last year, and 22% reported a fever which was also associated with headache and weakness. Fever accompanied by acute diarrhea was reported by 9% of participants, while 1% of people were admitted to hospital due to unexplained bleeding.

In DRC, biological samples and questionnaires were requested from eligible febrile patients in St. Joseph and Kingasani hospitals, but biological samples were not taken from community members in bushmeat markets and community settings.

PARTICIPANT CHARACTERISTICS ACROSS HOSPITAL & SURVEILLANCE SITES

	COMMUNITY-BASED SURVEILLANCE	CLINIC-BASED SURVEILLANCE	
SITES	KINSHASA MARKETS, BUTA, & OTHER INDEPENDENT SITE (n = 27)	ST. JOSEPH (n = 207)#	KINGASANI (n = 212)
GENDER			
FEMALE	7 (26%)	114 (55%)	66 (31%)
MALE	20 (74%)	93 (45%)	146 (69%)
AGE (YEARS)*			
<18	- (-)	47 (2 - 17)	58 (2-16)
≥18	27 (24 - 68)	159 (18 - 86)	154 (18 -85)

*Mean (range)

#No age data for one participant

KEY INSIGHTS

From our behavioral research, the following risk characterization insights were gleaned regarding bushmeat practices:

- Nearly all bushmeat butchers avoid cleaning their cutting/butchering utensils or the work surfaces with soap. Almost unanimously, they said that rinsing with water is sufficient, and a significant number said they do not rinse tools at all, since the taste of the bushmeat might be affected by the soap and water.
- Participants do believe cleaning the market work area is important, but practices vary widely. Market cleaning and rubbish disposal seem generally well-organized. Better access to soap and clean water, combined with educational messages, could have positive uptake.
- Many butchers and bushmeat vendors wear clothes for market work that are kept separate from home clothes. This use of dedicated clothing could be reinforced as a self-protective measure and serve as a means to discuss other PPE use. PPE such as gloves, masks, or proper footwear, were rarely used by bushmeat vendors or butchers.
- Few participants were aware of disease risks from slaughtering/butchering with an open wound, and most market workers simply rinse wounds with water and continue working. Education around this exposure risk is warranted. Proper wound care and treatment components should be taught to reduce exposure risk during butchering.
- Bushmeat is usually frozen overnight, but then left out to thaw in the daytime. With inconsistent and unreliable electricity in the market, and the fact that bushmeat often takes several days to sell, there is a public health risk related to the inadequate preservation of meat.

OUTREACH & RISK COMMUNICATION

Given the zoonotic disease risk associated with bat contact that was identified through the PREDICT project's global surveillance activities, we conducted community outreach campaigns in Kongo Centrale, focusing on villages where bats are a regular food staple and meat source, serving both as sustenance and income generation. The purpose of the visit to communities was to increase awareness and promote risk reduction strategies. Our team held a risk reduction campaign using materials developed by the PREDICT project, including PREDICT's behavior change communication and risk reduction resource *Living Safely with Bats.* Community members, both adults and children, participated in this event to learn about bats, their important contributions to ecosystem services, and strategies for reducing risk of exposure to bat-borne zoonoses, while ensuring conservation of the bats. The outreach focused most intensively on considerations for alternative protein and income sources, as people are very reliant on bats in these communities.

CAPACITY STRENGTHENING

For DRC nationals, one of the highlights of the PREDICT program was the laboratory trainings provided to broad groups of trainees including government staff, medical and veterinary students, and other health specialists, which significantly increased their lab skills. Not only did dozens of people learn the steps involved in preparing samples for testing, performing the testing, and interpreting results, they also learned about the proper management of a highly functional laboratory, which includes keeping inventory, sample workflow to avoid contamination, and preparing for maintaining sample integrity in the event of power outages. The guidance and mentorship provided by the PREDICT project in DRC has expanded the molecular diagnostic platform at the Institut National Recherche Biomédicale (INRB) to include screening for important known and new viral zoonoses enabled the expansion of available services and has provided the capability to allow for the identification Ebola virus by amplifying viral RNA, as was done at INRB in partnership with PREDICT, in both 2014 and 2017.

OUTBREAK RESPONSE & PREPAREDNESS

In the 10 years that the PREDICT project has been operating in DRC, there have been many opportunities for the team to assist with and contribute to outbreak investigation and response. During this time, the team assisted the DRC government with investigations of suspected zoonotic disease cases and outbreaks, including Ebola virus, yellow fever, an unknown viral hemorrhagic fever which was caused by the newly identified Bas Congo virus, and unknown bonobo illnesses. The PREDICT project has been able to aid in the response to outbreaks by providing diagnostic testing, technical capacity, and field equipment. For example, the PREDICT project supplied PPE, PCR equipment, and laboratory supplies for Ebola outbreaks and procured a mobile laboratory for the surveillance of Yellow Fever as the result of a large outbreak. That mobile laboratory has since been repurposed for use during Ebola outbreak response. More broadly, the PREDICT team has been actively training government, animal, and human health staff to identify, manage, and to control outbreaks, leaving the country more prepared than ever to respond to emerging disease threats.

LOCAL PARTNERS

- Central Veterinary Laboratory, Kinshasa
- Centre de Réhabilitation des Primates de Lwiro, South Kivu Province
- Gorilla Rehabilitation and Conservation Education Center, North Kivu Province
- Institut Congolais pour la Conservation de la Nature (ICCN)
- Institut National de Recherche Biomédicale (INRB), Kinshasa
- Mountain Gorilla Veterinary Project (MGVP, Inc.) aka Gorilla Doctors
- National Veterinary Laboratory, Goma
- Provincial Agriculture Departments (IPAPEL) of North and South Kivu, Goma and Bukavu
- Provincial Health Departments (IPS) of North and South Kivu, Goma and Bukavu
- Provincial Ministry of Environmental Conservation and Tourism, North Kivu Province
- School of Veterinary Medicine, University of Butembo
- School of Veterinary Medicine, University of Lubumbashi
- Union of Conservation for Gorilla Conservation and Community Development (UGADEC)

EDDY KAMBALE SYALUHA

Country Coordinator, *Gorilla Doctors (MGVP, Inc.)*

"In the course of implementing PREDICT in North and South Kivu, we were very often asked by park personnel, health clinic workers, and members of the community what was the purpose of our sampling efforts, as they had never seen anybody doing anything like it. It was heartening to see how people received PREDICT messages about the One Health approach to human health protection. Awareness is now so high that human health clinic staff now alert PREDICT about patients who had been bitten by wild animals."

JEAN PAUL LUKUSA KABEMBA

Human Surveillance Coordinator, *Gorilla Doctors (MGVP, Inc.)*

"As a medical technologist who has spent many years working with Gorilla Doctors to manage our Employee Health Program, I was extremely happy to help implement the PREDICT project in eastern DR Congo. It helped me better appreciate how closely people come into contact with wildlife in their daily lives, and I am happy to have helped people understand how they can protect themselves from zoonotic disease while also appreciating how important wildlife are in our world."

ACHIEVEMENTS

The world's second largest Ebola virus outbreak in history was declared in North Kivu Province in August 2018. The PREDICT project has played an important role in outbreak response and preparedness and has:

- Stockpiled outbreak investigation kits that are ready for deployment, should an outbreak spread to areas in and around Virunga National Park (PNVi).
- Led a workshop for 60 governmental laboratory technicians, district veterinarians, and public health workers on the importance of One Health approaches to disease outbreaks like Ebola virus, and conducted multiple awareness-raising meetings in and around PNVi to inform park personnel and their families about Ebola virus, the outbreak, and how to protect themselves from infection.
- Worked closely with partners to advise PNVi on how best to protect park workers and mountain gorillas from Ebola virus infection, and on best practices should there be a positive Ebola virus case in a person or gorilla.
- Participated in a training session along with government personnel (Ministry of Agriculture Animal Health Laboratory in Goma) and the Central Veterinary Laboratory of Kinshasa in collaboration with Université Pédagogique Nationale (UPN) on JICA protocols on Quick Ebola Virus Disease test.
- Worked with outbreak affected communities to raise awareness of zoonotic disease threats and strategies for risk reduction using the PREDICT project-developed resource *Living Safely with Bats* as an entry point for sharing health education and conservation messages.

ONE HEALTH SURVEILLANCE

From 2014-2019, the PREDICT project focused One Health surveillance at the wildlife-human interface in North and South Kivu Provinces. Our teams worked inside the Mikeno sector of Virunga National Park (PNVi) and in the adjacent communities, capturing and sampling primates and bats. In addition, our team enrolled consenting febrile patients (including park workers) presenting to local health clinics in our surveillance effort, collecting biological samples and administering questionnaires to better understand the social and behavioral risks for animal contact and zoonotic disease transmission. We also focused surveillance in and around Kahuzi-Biega National Park in South Kivu Province, home to the Centre de Réhabilitation des Primates de Lwiro, which receives and cares for confiscated and rescued wildlife, mainly great apes and other primates. In total, our team safely and humanely collected samples from 154 non-human primates, 288 bats, and 12 rodents, and together with local health centers, we enrolled and collected samples from 416 febrile human patients.

Samples from wildlife and humans were safely tested using consensus PCR (cPCR) to screen for priority zoonotic viral diseases and emerging threats such as filoviruses (e.g. Ebola and Marburg), influenzas, coronaviruses (e.g. SARS and MERS), paramyxoviruses, and flaviviruses. Virus findings were confirmed through genome sequencing and interpreted to better understand the relationship of the detected sequence to those from known animal and human pathogens. Virus testing of both animal and human samples was performed at the Institut National de Recherche Biomédicale (INRB) in Kinshasa and at the University of California, Davis One Health Institute (OHI) Laboratory.

Two known viruses that cause respiratory illnesses were detected in seven people from North Kivu at the clinic serving the Virunga National Park area: Influenza A virus (n=2) and Betacoronavirus 1 (OC43) (n=5). Three of the seven positive individuals were children under the age of eight; the other four positive individuals were adults between the ages of 19 and 34.

Betacoronavirus 1 (OC43), or human coronavirus OC43 (HCoV-OC43), was detected in oral swab samples from two children and three adults, in both males and females from the two age groups. HCoV-OC43 is one of the known viruses that causes the common cold, as well as severe lower respiratory tract infections, and can cause pneumonia in infants, the elderly, and immunocompromised individuals such as those with HIV/AIDS.

Influenza A was detected in one female child and one adult male. Both of the positive detections came from oral swab samples.

There were no virus findings from any of the tested wildlife samples.

SURVEILLANCE SITES

NORTH & SOUTH KIVU PROVINCES

bats; humans;
non-human primates;
rodents/shrews

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS
Coronavirus	Betacoronavirus 1 (OC43)	Human	Clinic (Virunga National Park)	5
Influenza virus	Influenza A	Human	Clinic (Virunga National Park)	2
Total				**7**

OUTREACH & RISK COMMUNICATION

COMMUNITY OUTREACH

Through One Health surveillance, the PREDICT team in North and South Kivu engaged local communities and experienced a very positive attitude and acceptance among community members and park employees. Community members stated that it was very important to know about potential risks for contracting diseases from wildlife given their high level of contact – whether from utilizing shared water sources in bat caves or wetlands frequented by forest buffaloes, fending off baboons, chimpanzees, and gorillas feeding on their crops, or sharing their own dwellings with bats.

Our team was active during the Ebola virus outbreak in North Kivu and Ituri Provinces, and in general we found that people realized wildlife pose a potential health threat, and that there are actions they can take to protect themselves. By engaging at-risk communities, the PREDICT team shared the risk reduction and behavior change communication resource, *Living Safely With Bats*, and used the resource an entry point for explaining to people that they can take steps to avoid contact with bats, such as avoiding hunting, bushmeat trading, and wildlife trafficking, while also allowing bats to continue to play their vital roles in our ecosystem as pollinators and seed dispersers, and in controlling insect populations.

DEMOCRATIC REPUBLIC OF THE CONGO PRACTICAL IMPLICATIONS

The PREDICT project's work in DRC has been pivotal in establishing the premise of a One Health platform in-country. While some of the world's foremost experts on Ebola response are based in DRC, the PREDICT project's family-level assays brought important PCR diagnostic confirmation at a crucial time, helping to quickly provide real-time solutions in the face of an Ebola outbreak. The PREDICT project has brought into focus the importance of zoonotic disease spillover in the Congo Basin, one of the hotspots of infectious disease emergence. With its emphasis on capacity expansion, the PREDICT team has reinforced human and animal health preparedness and response capabilities in the country and increased awareness among communities of the potential for spillover of wildlife viruses. Because of close collaborative ties across the PREDICT Consortium, Congolese health experts now have regional colleagues that they reach out to for consultation and collaborative reinforcement. This rich multidisciplinary, transborder partnership established by the PREDICT project will persist, we hope, in sustaining the vigilant surveillance of zoonotic disease risk in DRC and throughout the region.

The PREDICT project has provided technical trainings for dozens of government workers, health and medical staff, veterinary and wildlife workers, and for students who will become the next leaders in global health. For nearly a decade, the DRC team has also performed vital services such as assisting with disease outbreak investigations, conducting studies assessing risk associated with bat guano farming and tourist interactions with bats, performing follow-up studies of Ebola virus exposure in outbreak zones, creating opportunities for enhancing awareness about zoonoses in bushmeat markets and with wildlife hunters, and spreading the knowledge gained by this program across the world through both peer reviewed publications and presentations at international conferences. The seeds have been planted and the DRC efforts will continue to grow from the strong roots that USAID has helped nurture through the PREDICT project.

As we say in French, Lingala, Kiswahili and English:

L'equipe restera forte!
Toko koba makasi!
Timu itabaki kuwa imara!
The team will stay strong!

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SPECIAL FEATURES

EBOLA OUTBREAK IN MBANDAKA

In May of 2018, the DRC province of Equateur experienced an outbreak of Ebola virus – its second outbreak following the one in 2014. It isn't known where the virus originated but is was suspected that bushmeat may be to blame. To learn more about this outbreak and how people could possibly have become infected, PREDICT/DRC dispatched a team to the capital city of Equateur, Mbandaka, to dig deeper into the mystery.

To learn more about this study: **bit.ly/39NMsRJ**

ONE HEALTH PREPAREDNESS FOR EBOLA VIRUS DISEASE OUTBREAKS

Given the potential for Ebola virus to infect habituated mountain gorillas and cause devastating losses of this endangered species, the PREDICT eastern DRC team took action to build greater awareness of Ebola virus as a One Health pathogen among health professionals and in communities, using these outreach efforts to educate people on the virus and how to minimize risk for infection. The PREDICT team convened an all-day workshop involving veterinarians, public health workers, and laboratory technicians, educating attendees about animals as sources of human disease and the role of veterinarians in helping protect human health. They used the Ebola outbreak as impetus for reviewing hygiene practices that help minimize infectious disease transmission. A similar training was held for people working in national parks and forest reserves adjacent or near to the EVD outbreak zone.

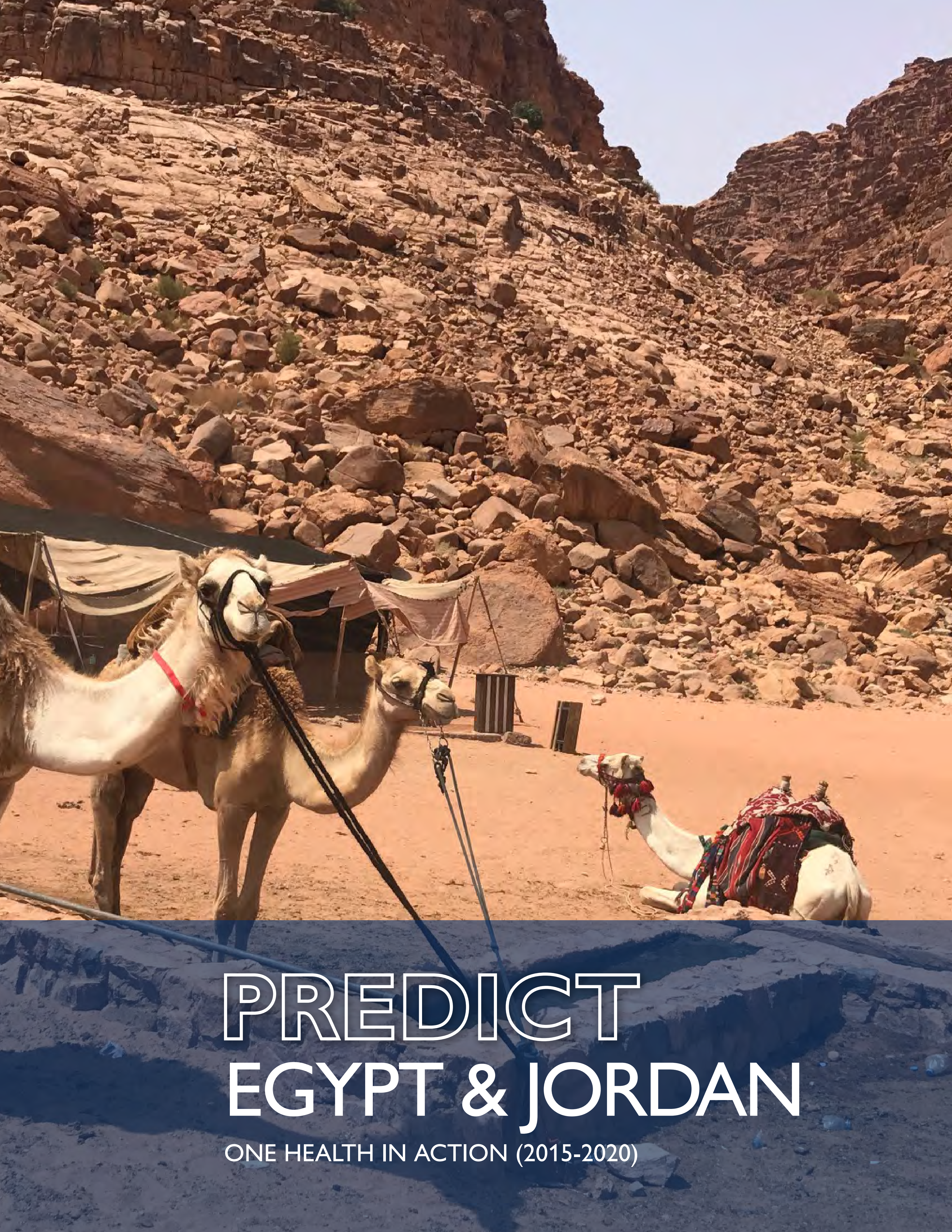
To learn more about this study: **bit.ly/39Oql8s**

SPECIAL FEATURES

EVIDENCE FOR EXPOSURE TO EBOLAVIRUSES PRIOR TO THE 2018 OUTBREAK

The second largest Ebola virus disease (EVD) outbreak began in North Kivu Province in eastern DRC in July 2018 and soon spread to neighboring Ituri Province. Concurrently, another outbreak affected Equateur Province in western DRC. Data suggest that these outbreaks were not epidemiologically linked, and that independent introduction of Ebola virus (EBOV) into humans had occurred in both areas. Therefore, we conducted serologic testing for exposure to ebolaviruses in febrile patients seeking care in North Kivu Province prior to the EVD outbreak. Serum samples from 272 indivduals (152 adults and 120 children, of which 164 were female and 108 were male) that presented with fever to the Rubare Health Center (in the Rutshuru Health Zone) before the start of the EVD outbreak were analyzed at the OHI lab. Of the samples analyzed, 30 were reactive on initial screening against Ebola virus, and of those, 29 were determined to be positive for antibodies to *Zaire ebolavirus* and one for antibodies to the newly detected *Bombali ebolavirus* (BOMV; Goldstein et al., 2018). Positives were equally distributed among adults (11%, 16/152) and children (12%, 14/120); more females were positive (14%, 23/164) than males (6%, 7/108). Most illuminating, seropositive people were detected even in the months prior to the current 2018 North Kivu/Ituri Ebola virus outbreak. This is also the first documented evidence of exposure to ebolaviruses in people in eastern DRC and the first known detection of antibodies to BOMV, previously detected in bats in West and East Africa, which shows that human exposure to BOMV has occurred. Our data suggest human exposure to ebolaviruses may be more frequent and geographically widespread than previously thought.

Adapted from Goldstein et al. 2020. Spillover of ebolaviruses into people in Eastern Democratic Republic of the Congo prior to the 2018 Ebola Virus Disease Outbreak. In review.



PREDICT
EGYPT & JORDAN

ONE HEALTH IN ACTION (2015-2020)

Surveillance for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in North Africa and the Middle East

Globally, there have been more than 2,250 confirmed human cases of Middle East respiratory syndrome (MERS) coronavirus causing more than 800 deaths. The majority of human MERS infections have been detected in the Middle East, including the first known human cases in Jordan. Dromedary camels are the presumptive source of primary human MERS infections. The PREDICT project in Egypt and Jordan worked with existing public and animal health surveillance systems by exploring the incidence of priority virus groups in rarely studied hosts such as bats and camels. PREDICT Egypt and Jordan teams played an integral part in the study of MERS-CoV at the animal-human interface by looking at evidence of MERS infection in camels, humans exposed to camels, and bats. Together with host country governments and our local partners, we also looked into viruses that are not commonly studied as part of the routine public and animal health surveillance in Jordan or Egypt such as filo- and paramyxoviruses.

In Egypt, there are significant camel-human interfaces, and dromedary camel herds in Greater Giza were found to have evidence of infection with MERS coronavirus. These sites are also high tourist areas where camels are used for tourists to ride or have their pictures taken with the animals. In Al Khatatbah, a natural area in Egypt, there are significant bat populations which are distributed directly along camel trade routes connecting camel markets to camel abattoirs.

In collaboration with Food and Agriculture Organization (FAO) of the United Nations, the PREDICT team in Jordan investigated zoonotic spillover risk at interfaces between humans, bats, and camels. Working with ministry partners and FAO, sites along the camel value chain such as abattoirs, live animal markets, and farms were identified for biological surveillance and behavioral risk characterization. The first known human cases of MERS were in Middle Jordan, and through our work we found camel herds in Middle Jordan with MERS virus. Camel herds in Northern Jordan were also found to have MERS virus.

EGYPT

Global Health Security Agenda

18

DEVELOPED the One Health Workforce by training 18 people in Egypt.

>2.1K

OPERATIONALIZED One Health surveillance and sampled over 2.1K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Center of Scientific Excellence for Influenza Viruses

19,117
TESTS

6
PREDICT

5
KNOWN

DETECTED 11 unique viruses in both animal and human populations.

AHMED KANDEIL
Researcher, *National Research Centre*

"PREDICT enhanced our lab capacity by making use of the virus testing protocols."

MOKHTAR GOMAA
Assistant Researcher, *National Research Centre*

"I enjoyed the multiple field trips collecting samples from bats and humans."

ACHIEVEMENTS

- Conducted surveillance on bats and applying the PREDICT influenza testing protocols on the collected samples led to the discovery of a new influenza virus in Egyptian fruit bats. This virus was isolated and characterized rendering it the first and only wildtype bat flu isolate globally. The virus was distinct from any previously discovered influenza A virus suggesting that it is potentially a novel H19N12 subtype.
- Close coordination and cooperation between PREDICT Egypt and PREDICT Jordan was mutually beneficial. Members of PREDICT Jordan visited the laboratory in Cairo to familiarize themselves with the work process. Members of PREDICT Egypt assisted with training the Jordanian team members on the conduct of human subject research. The lab in Cairo assisted PREDICT Jordan by conducting serological analysis for MERS-CoV antibodies on human sera collected in Jordan.
- Trained and used PREDICT laboratory diagnostic protocols to expand the lab's capacity beyond influenza and coronavirus testing to include paramyxovirus, flavivirus, and filovirus capacities.

LOCAL PARTNERS

- Ministry of Health
- Ministry of Environment

JORDAN

>20

DEVELOPED the One Health Workforce by training more than 20 people in Jordan.

>2.1K

OPERATIONALIZED One Health surveillance and sampled over 2.1K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Jordan University of Science and Technology

>49,395
TESTS

5
PREDICT

8
KNOWN

DETECTED 13 unique viruses in both animal and human populations.

EHAB ABU-BASHA

Jordan University of Science & Technology (JUST)

"PREDICT has provided me with an opportunity to become deeply involved in zoonotic disease research and health capacity building in Jordan. Our contributions to the PREDICT project will have a lasting impact on One Health implementation in Jordan."

MUSTAFA ABABNEH

Jordan University of Science & Technology (JUST)

"Discovering whether a detected virus has the potential to infect humans and to cause illness is a central part of the project. We may find a multitude of viral RNA in our samples, so prioritizing which findings are of greater importance helps us sort through the data."

ACHIEVEMENTS

- Trained 25 individuals, including two graduate students in One Health skills
- Sampled >2,100 individuals (animals and people)
- Interviewed >1,000 people on behavior and practices associated with viral transmission and spread, specifically MERS-CoV
- Strengthened one major research lab and two additional labs critical for surveillance and detection of zoonotic disease threats
- Detected six unique viruses and conducted the first ever reporting to OIE of MERS-CoV in camels
- Empowered ministry partners to establish and operationalize Jordan's first ever national One Health platform

LOCAL PARTNERS

- Food and Agriculture Organization (FAO)
- Jordan Ministry of Agriculture
- Jordan Ministry of Environment
- Jordan Ministry of Health
- Jordan University of Science and Technology
- Princess Haya Biotechnology Center
- Royal Scientific Society
- The Hashemite Fund for Development of Jordan (Badia Fund)
- The Royal Society for the Conservation of Nature
- USAID/Jordan
- World Health Organization (WHO)
- World Organization for Animal Health (OIE)

VIRUS DETECTION

The PREDICT project's strategy for virus detection included screening samples using broadly reactive consensus PCR (cPCR) for priority virus groups, including coronaviruses, filoviruses, flaviviruses, paramyxoviruses, and influenza virus. Positives detected using these assays were sequenced to identify the viruses and compare their relationship to known pathogens, and viruses were prioritized for further characterization. This approach allows for detection of both known and novel viruses and improves our understanding of the presence and diversity of viruses, as well as potential pathogens, in humans and animals.

In Egypt, PREDICT project protocols were implemented at the laboratory of the Center of Scientific Excellence for Influenza Viruses, part of the Egyptian National Research Centre in Giza. This is a governmental virology lab which specializes in conducting research on emerging viruses at the animal-human interface, particularly coronaviruses and influenza viruses. Sampling efforts led to the accumulation of more than 6,700 biological samples safely collected from both humans and bats. After conducting more than 17,000 tests on 2,388 bat samples and 363 human nasal swab samples, the team detected 11 unique viruses, all within bat hosts. These include five known viruses (three coronaviruses, one paramyxovirus, and one influenza virus) and six novel paramyxoviruses.

In Jordan, the PREDICT project's partner laboratory at Jordan University of Science and Technology (JUST) tested animal and human samples for coronaviruses to capture the virus diversity present in the family that includes MERS-CoV, in addition to testing for the other priority virus groups. Our team established a biobank of over 15,000 (>7,500 human and >7,500 bat) samples including whole blood, oral and nasal swabs, urine, feces, and serum. Over 16,000 PCR tests were conducted, resulting in the detection of 13 unique viruses (eight known coronaviruses, four novel coronaviruses, and one novel paramyxovirus).

VIRUS TABLE (EGYPT)

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	Bat coronavirus HKU9	Egyptian Fruit Bat	Al Khatatbah, Banha	9	9	0
	Rousettus bat coronavirus /NRC-1	Egyptian Fruit Bat	Banha	4	4	0
	Rousettus bat coronavirus /NRC-2	Egyptian Fruit Bat	Al Khatatbah, Banha	8	8	0
Paramyxovirus	PREDICT_PMV-113	Egyptian Fruit Bat	El Mansoria	1	0	1
	PREDICT_PMV-114	Egyptian Fruit Bat	Banha	1	1	0
	PREDICT_PMV-115	Egyptian Fruit Bat	Banha	1	1	0
	PREDICT_PMV-116	Egyptian Fruit Bat	Banha	1	1	0
	PREDICT_PMV-118	Egyptian Fruit Bat, Kuhl's Pipistrelle	Birqash, El Mansoria	14	0	14
	PREDICT_PMV-119	Egyptian Slit-Faced Bat	El Mansoria	1	0	1
	Bat paramyxovirus R_aeg_UPE766/525/122	Egyptian Fruit Bat	Al Khatatbah	1	1	0
Influenza virus	Influenza A	Egyptian Fruit Bat, Egyptian Tomb Bat, Kuhl's Pipistrelle	Al Khatatbah, Banha, Birqash, El Mansoria, El Shohada	157	116	41
Total				**198**	**141**	**57**

VIRUS TABLE (JORDAN)

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Aljoun, Wadi Alseer	5	5	0
	PREDICT_CoV-65	Greater Horseshoe Bat, Lesser Mouse-Tailed Bat, Mediterranean Horseshoe Bat, Mehely's Horseshoe Bat, Schreiber's Long-Fingered Bat, Unidentified Insectivorous Bat	Aljoun, Wadi Alseer	14	3	11
	PREDICT_CoV-91	Unidentified Insectivorous Bat	Aljoun	1	0	1
	PREDICT_CoV-112	Mehely's Horseshoe Bat	Aljoun	1	1	0
	PREDICT_CoV-113	Unidentified Insectivorous Bat	Aljoun	1	1	0
	Bat alphacoronavirus/ GS2013/HuB2013	Blasius's Horseshoe Bat, Egyptian Fruit Bat, Lesser Mouse-Tailed Bat, Mediterranean Horseshoe Bat, Mehely's Horseshoe Bat, Unidentified Bat, Unidentified Insectivorous Bat	Aljoun, Wadi Alseer 54	13	41	
	Bat coronavirus 1	Unidentified Bat	Aljoun	1	1	0
	Bat coronavirus BM48-31/ BGR/2008	Chinese Rufous Horseshoe Bat, Egyptian Fruit Bat, Lesser Mouse-Tailed Bat, Mediterranean Horseshoe Bat, Mehely's Horseshoe Bat, Schreiber's Long-Fingered Bat, Unidentified Bat, Unidentified Insectivorous Bat, Unidentified Rhinopomatidae Bat	Aljoun, Wadi Alseer	52	16	36
	Bat coronavirus HKU9	Egyptian Fruit Bat, Lesser Mouse-Tailed Bat, Mediterranean Horseshoe Bat, Unidentified Insectivorous Bat	Aljoun, Wadi Alseer	6	4	2
	Betacoronavirus 1 (Bat)	Unidentified Insectivorous Bat	Aljoun	1	0	1
	Rousettus bat coronavirus/ NRC-1	Egyptian Fruit Bat, Unidentified Insectivorous Bat	Aljoun, Wadi Alseer	3	3	0
	Rousettus bat coronavirus/ NRC-2	Egyptian Fruit Bat	Wadi Alseer	2	2	0
Paramyxovirus	PREDICT_PMV-176	Lesser Mouse-Tailed Bat	Wadi Alseer	1	0	1
Total				**138***	**48***	**90***

*Numbers do not total as four bats were co-infected with two viruses

FINDINGS IN PEOPLE

The PREDICT team in Egypt safely tested 2,541 nasal swab samples from humans, an effort which resulted in no positive virus detections. These results indicate that none of the participants sampled had active infections at the time, but do not preclude the possibility for previous exposure to viruses, as the PREDICT project's cPCR assays are only capable of detecting active viral infections.

In Jordan, the only virus detected in humans (n=5) was betacoronavirus 1 (OC43), a human coronavirus known to cause mild to moderate respiratory illness. Although belonging to the same genus as SARS-CoV-2, the novel betacoronavirus causing the current COVID-19 pandemic, OC43, is not closely related to SARS-CoV-2. OC43 is an established human coronavirus first detected in the 1960s and one of seven coronaviruses known to infect humans (229E, NL63, OC43, HKU1, SARS, SARS-2, and MERS).

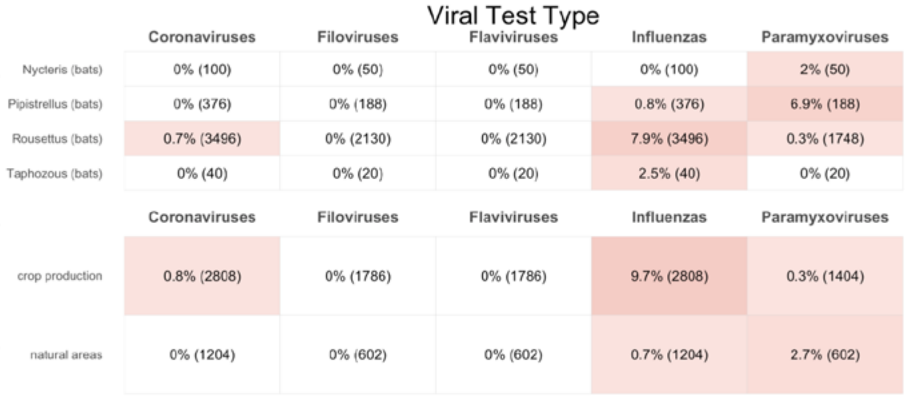
FINDINGS IN BATS

In total, the PREDICT team in Egypt tested 2,388 bat samples (oral, rectal, and oropharyngeal swabs, and blood) collected from 1,003 individual bats. One hundred ninety eight (198) of these specimens tested positive for a coronaviruses, paramyxoviruses, or influenza viruses. Paramyxovirus diversity was the highest, as seven unique viruses were detected in three different bat species. A novel paramyxovirus, PREDICT_PMV-118, was detected in 14 individuals; one frugivorous bat, the Egyptian fruit bat (*Rousettus aegyptiacus*), and 13 insectivorous bats, Kuhl's pipistrelle (*Pipistrellus kuhlii*). All other detected paramyxoviruses were only found in one individual, each. Although PREDICT_PMV-118 was the most commonly detected paramyxovirus by the PREDICT team in Egypt, there is no evidence to suggest that this virus, nor any other paramyxovirus detected, pose a threat to human health.

All three coronaviruses detected are known bat viruses and were found within a single species, the Egyptian fruit bat, from two independent sites. Each of the 21 coronavirus detections occurred during the wet season despite a relatively even sampling effort across the wet and dry seasons at Al Khatatbah and Banha. Although just a single influenza virus, influenza A, was detected within bats, it was the most commonly detected virus (infecting 157 individual bats), infected the greatest diversity of bat hosts (three species; fruit-eating and insect-eating), and was the most widely distributed (found at five independent sites). In addition, influenza A was more commonly detected in the wet season (74%) than dry season (26%).

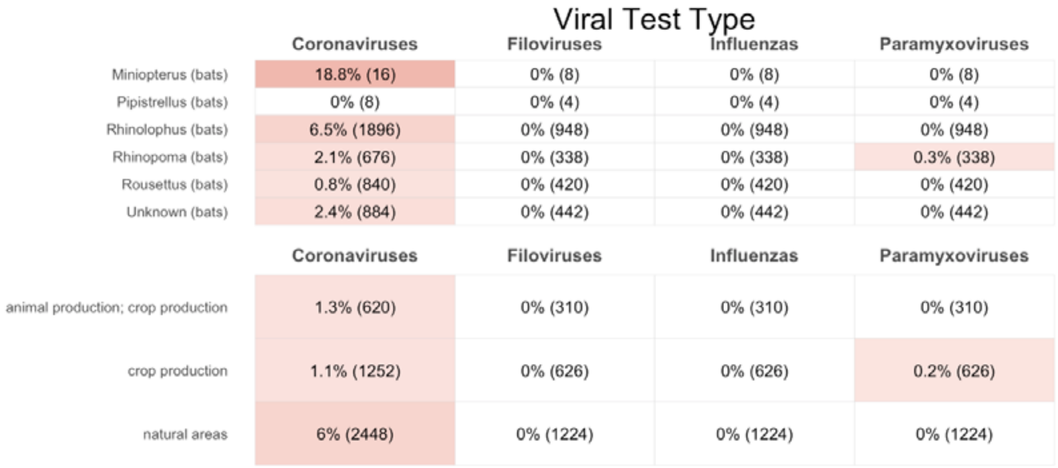
A similar testing effort from the PREDICT team in Jordan for bats was observed; 2,160 bat samples representing 1,080 unique bats were analyzed, of which 133 individuals were positive for coronavirus and/or paramyxovirus infections. Twelve unique viruses (11 coronaviruses and one paramyxovirus) were detected in at least eight bat species from two sites in northwestern Jordan. Of the four novel coronaviruses detected, none are believed to pose a threat to human health. The other seven coronaviruses detected are known bat coronaviruses belonging to the alphacoronavirus and betacoronavirus genera, of which three betacoronaviruses were also detected in Egypt in the same species, Egyptian fruit bats.

Three individual insect-eating bats from Aljoun belonging to the *Rhinolophus* genus were co-infected with two coronaviruses, bat alphacoronavirus/GS2013/HuB2013 and bat coronavirus BM48-31/BGR/2008. These two known coronaviruses were also the most commonly detected viruses in Jordan, together representing approximately 75% of the total virus detections in bats. An additional *Rhinolophus* sp. from this site was also co-infected with two coronaviruses, the known bat coronavirus BM48-31/BGR/2008 and novel PREDICT_CoV-113. The single paramyxovirus detected was a novel virus, PREDICT_PMV-176, in one lesser mouse-tailed bat (*Rhinopoma hardwickii*). This is the sole detection of PREDICT_PMV-176 in the entire PREDICT project, suggesting that this is not a prevalent virus.


Viral Test Type

	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
Nycteris (bats)	0% (100)	0% (50)	0% (50)	0% (100)	2% (50)
Pipistrellus (bats)	0% (376)	0% (188)	0% (188)	0.8% (376)	6.9% (188)
Rousettus (bats)	0.7% (3496)	0% (2130)	0% (2130)	7.9% (3496)	0.3% (1748)
Taphozous (bats)	0% (40)	0% (20)	0% (20)	2.5% (40)	0% (20)

	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
crop production	0.8% (2808)	0% (1786)	0% (1786)	9.7% (2808)	0.3% (1404)
natural areas	0% (1204)	0% (602)	0% (602)	0.7% (1204)	2.7% (602)



Viral Test Type

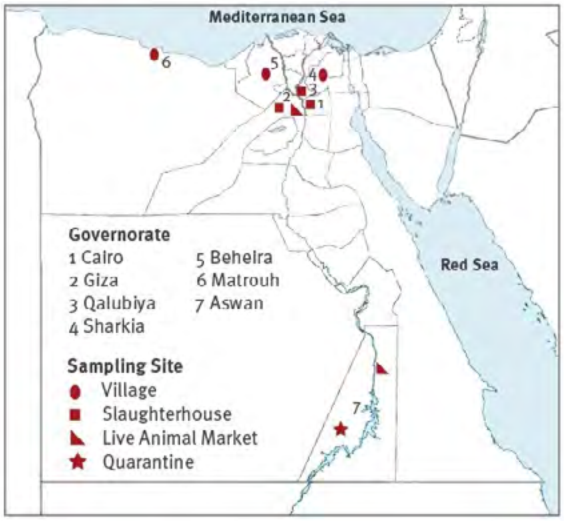
	Coronaviruses	Filoviruses	Influenzas	Paramyxoviruses
Miniopterus (bats)	18.8% (16)	0% (8)	0% (8)	0% (8)
Pipistrellus (bats)	0% (8)	0% (4)	0% (4)	0% (4)
Rhinolophus (bats)	6.5% (1896)	0% (948)	0% (948)	0% (948)
Rhinopoma (bats)	2.1% (676)	0% (338)	0% (338)	0.3% (338)
Rousettus (bats)	0.8% (840)	0% (420)	0% (420)	0% (420)
Unknown (bats)	2.4% (884)	0% (442)	0% (442)	0% (442)

	Coronaviruses	Filoviruses	Influenzas	Paramyxoviruses
animal production; crop production	1.3% (620)	0% (310)	0% (310)	0% (310)
crop production	1.1% (1252)	0% (626)	0% (626)	0.2% (626)
natural areas	6% (2448)	0% (1224)	0% (1224)	0% (1224)

Building from Table 1 (the virus detection tables above), these heatmaps provide more granular information about sampling effort and detection rates across the five priority virus groups: coronaviruses, filoviruses, flaviviruses, influenza viruses, and paramyxoviruses. First, data are summarized at the host genus level, and broader taxonomic groups are shown in parentheses. Next, data are summarized by reported disease transmission interfaces at the sampling sites. Data within the heatmaps show the percentage of virus detections that were confirmed positive (%) and the number of PCR tests conducted (in parentheses). The intensity of red color within the heatmaps scales with virus detection rate. Host taxa or interfaces that never yielded positive virus tests for a given virus group are shown in white, while red coloration indicates increasingly higher rates of virus detection.

ISOLATION & CHARACTERIZATION OF A DISTINCT INFLUENZA A VIRUS FROM EGYPTIAN FRUIT BATS

As part of surveillance efforts, the PREDICT project safely collected samples from four bat species in Egypt. Samples were tested for influenza virus and our team detected, isolated, and characterized a novel subtype of influenza A virus in Egyptian fruit bats. Out of 1,202 swabs analyzed, 105 swabs collected from a single roosting site tested positive by real time PCR (RT-PCR). The roosting site was in an abandoned house within a village in a densely inhabited agricultural area in the Nile Delta region. Bats from the roost likely fed on fruits in nearby village orchards. Genetic analysis of the virus suggested ancestry with other H9 viruses, and the virus showed a low level of cross-reactivity with serum raised against H9N2 viruses. The virus had an affinity to avian-like receptors and may have originated from an avian host. In controlled laboratory experiments, the virus was also shown capable of infecting mice. To explore exposure to the virus in bats in the roost, we collected additional serum a few weeks after the initial detection of the virus. Antibody testing showed that bats were seropositive for the isolated viruses, though no signs of disease were noted in the roost. Viruses such as this novel subtype of influenza may have the potential to infect across taxa, including domestic birds, mammals, and potentially humans. We recommend further surveillance in bats for disease threats to better understand the distribution, diversity, and potential risks of spillover. This work was published in the Journal of Virology in January 2019 (Kandeil et al., 2019).



Map of the collected samples from dromedary camels and domestic animals in Egypt. Credit: Ali et al., 2017, EuroSurveillance.

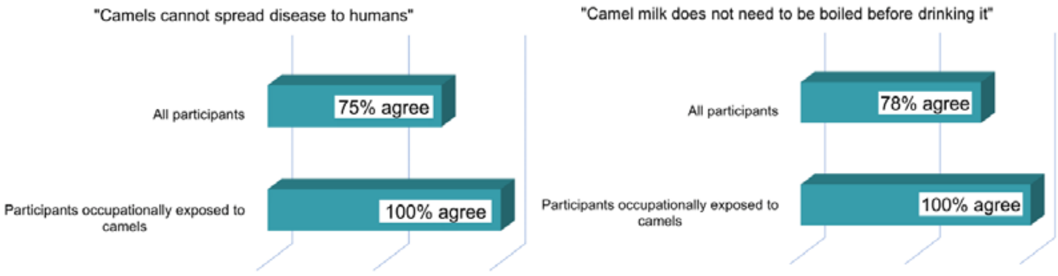
EPIDEMIOLOGICAL & BEHAVIORAL RISK

TRANSBOUNDARY MERS-CoV RISK ALONG THE CAMEL-MARKET VALUE CHAIN

Our team in Egypt partnered with FAO to investigate the prevalence of MERS-CoV in imported and resident camels along with the prevalence of the virus among other domestic animals in country. Between August 2015 and January 2016, 1,176 sera and 1,223 nasal swabs were collected from 1,223 animals: 1,078 dromedary camels (339 resident and 739 imported) and 145 other domestic animals (cattle, sheep, goats, donkeys, buffalo, and horses) from quarantine posts, live animal markets, slaughterhouses and villages across seven governorates of Egypt. Samples were also collected from 24 fruit bats (*Rousettus aegyptiacus*) and 85 insectivorous bats (*Pipistrellus kuhlii, Nycteris thebaica,* and *Taphozous perforates*) from Abo Rawash, Giza governorate. Serologic and molecular testing for MERS-CoV was performed on the samples using serological neutralization assays and real-time reverse transcription-PCR (rtRT-PCR). Of the 1,031 camels serologically tested, 871 (84.5%) had MERS-CoV neutralizing antibodies in their sera and the seroprevalence was significantly higher in imported (614/692; 88.7%) than in resident camels (75.8%). Seroprevalence varied significantly among camels originating from East Africa, Sudan, and Egypt. Additionally, we found significant differences between camels sampled from live animal markets, quarantine facilities, slaughterhouses, and villages. Adult camels had significantly higher seroprevalence (87.3%) than young camels (51.8%). Forty-one (3.8%) camels tested positive for MERS-CoV using MERS-CoV PCR tests, indicating the presence of active or passive viral infection. Four of these camels originated from East Africa, 35 from Sudan, and the other two from the study sites in Egypt. None of the 91 tested bats were positive for MERS-CoV neutralising antibodies and all genetic tests performed on bat samples were also negative. This study clearly shows that MERS-CoV is ubiquitous in camels in Egypt and highlights the role camels play as intermediate hosts for MERS-CoV especially in the transboundary camel value chain. While no human cases have been reported from the countries of origin in Sudan and East Africa, additional surveillance and monitoring is critical to follow camels from their country of origin until they reach the slaughterhouses to better understand the risks and epidemiology of the disease along the camel market chain.

KNOWLEDGE, ATTITUDES, BELIEFS, AND PRACTICES PERTAINING TO CAMEL-TO-HUMAN DISEASE RISKS IN JORDAN

Even the most efficacious behavioral interventions require overcoming knowledge, attitudinal, belief, cultural, and practical barriers in order to be effective, sustainable, and successful. In Jordan, the PREDICT team worked with a population occupationally exposed to camels to characterize the knowledge, attitudes, and beliefs pertaining to camel-to-human disease risks and to identify potential barriers to implementing protective practices such as use of personal protective equipment (PPE) and frequent handwashing. From February 2018 to August 2018, 755 participants were enrolled and interviewed using a focused questionnaire designed to assess knowledge, attitudes, and beliefs about camel-to-human disease risks. For those reporting working with camels or camel products, or within camel living environments, another interview was conducted using a separate questionnaire assessing PPE and handwashing practices as well as attitudes and beliefs about using various types of PPE at work.



Note: All participants n=755; participants occupationally exposed to camels n=38

FIGURE 2. *Knowledge, attitudes & beliefs on camel-to-human disease risks in Jordan*

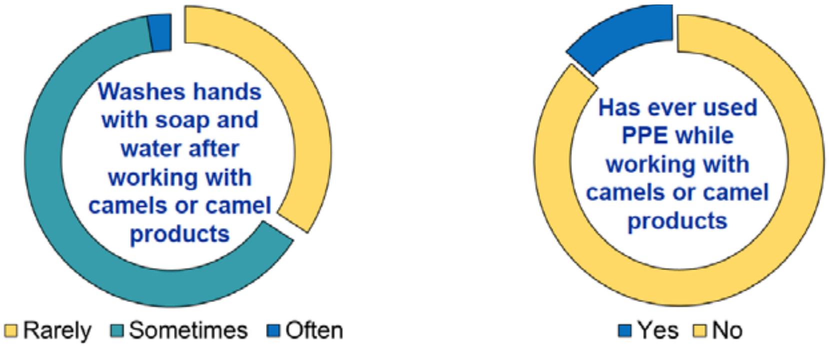


FIGURE 3. *Self-reported biosecurity behavioral practices among those occupationally exposed to camels in Jordan*

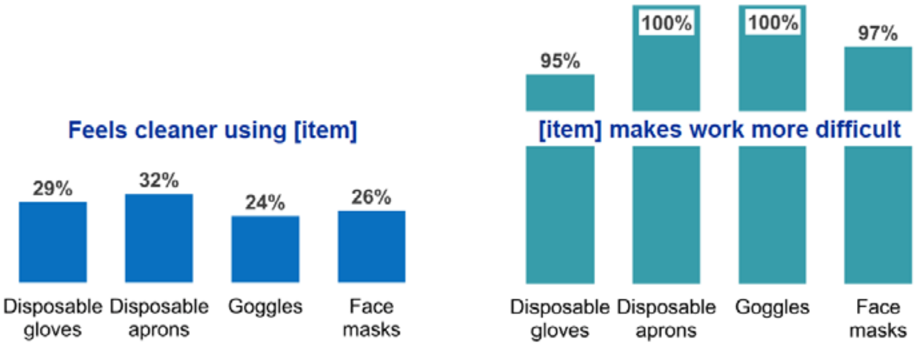


FIGURE 4. *Attitudes and beliefs about PPE use among those occupationally exposed to camels in Jordan*

Our results clearly show barriers to implementing protective practices that could reduce risks for disease transmission in at-risk individuals that have frequent contact with camels. In this population there is a widespread belief that camels cannot spread disease to humans, particularly among those who are routinely occupationally exposed to camels. Current PPE use and frequent handwashing among those working with camels is minimal, and use of PPE such as gloves, protective clothing, and face coverings is widely perceived as burdensome. We recommend addressing these barriers through a multifaceted risk reduction and behavioral change communication intervention that promotes knowledge about camel-to-human disease risk, provides PPE and handwashing stations, provides training on PPE and handwashing practices, and normalizes PPE use and handwashing among those working with camels or camel products.

> "Your efforts in Jordan to track and monitor emerging viruses kept us all safer in an ever-changing world of infectious disease risk. I admire your team's dedication to this critically important mission. Time and again, I saw the excellent relationships you built with counterparts and communities to expand our global knowledge of disease risk. You and the team exemplified some of USAID's very best work, and I am honored to have played a part in this strong partnership."
>
> –Andrea Halverson
> Deputy Director,
> *Population & Family Health Office, USAID Jordan*

CAPACITY STRENGTHENING

As part of the PREDICT project's strategic regional south-south training exchanges to share knowledge and develop communities of practice, we promoted collaboration between Egypt's National Research Centre's Center of Scientific Excellence for Influenza Viruses and the Jordan University of Science and Technology through joint training and sample/data sharing. The PREDICT Egypt team traveled to Jordan for hands-on training in safe bat capture and sampling. In turn, the PREDICT Jordan team assisted with safe and effective implementation of One Health surveillance in at-risk Jordanian communities. In addition, PREDICT lab teams worked together to better understand exposure to Middle East respiratory syndrome coronavirus (MERS-CoV). Human samples from both countries were screened for MERS-CoV-neutralizing antibodies and data was shared across institutions. This allowed for regional insights on MERS exposure in the North Africa and Middle East regions.

The PREDICT team in Egypt received rigorous laboratory and field training as part of their involvement in project activities. Many were early career stage scientists that significantly benefited from the training. PREDICT's work on MERS assisted both the animal and public health sectors to detect MERS-CoV in camels in Egypt thus providing critical information in real time as testing of the received samples was completed within a very short period of time and data shared as soon as they were compiled. Through PREDICT project investments, a total of 18 individuals were trained (nine men and nine women) in Egypt, all were members of the National Research Center.

In Jordan, the PREDICT project played a critical role in the development of a state-of-the-art molecular and virology laboratory at the Faculty of Veterinary Medicine-JUST. The JUST Molecular and Virology Laboratory is now considered an important hub for training and preparing future scientists as many graduate students are being trained in the laboratory to perform various techniques from safe sample handling to DNA extraction, cDNA synthesis, performing different PCR protocols, cloning, plasmid purification, and sequence analysis. The team also benefited from the hard work of two Master's graduate students (Ola Ababneh and Ghadeer Alzghoul) who worked with PREDICT project scientists as part-time research assistants in the laboratory. Ola and Ghadeer earned their Master's degrees and continue to work with the project's JUST team in technical support roles. Through PREDICT project investments, a total of 25 individuals were trained in Jordan, including 10 students, two staff members from the Ministry of Agriculture, and one representative from FAO.

Our team in Jordan also strengthened disease detection at the Ministry of Agriculture Veterinary Services by providing needed infrastructure and training to two diagnostic laboratories in the South of Jordan (Ma'an and Karak). Both laboratories were equipped with biosafety cabinets, hematology and chemistry analyzers, ELISA readers and washers, incubators, centrifuges and microcentrifuges, balances, microscopes, autoclaves, and other essential tools to strengthen disease detection capacity and stand ready to tackle disease threats such as COVID-19.

PRACTICAL IMPLICATIONS

Egyptian and Jordanian institutions have the capacity and capabilities to effectively conduct surveillance and identify risk factors for infectious disease emergence and contribute to pandemic prevention at national and regional scales that can now be utilized and expanded upon by national authorities and other stakeholders.

More than 30 early and mid-career professionals received long-term training and support in surveillance, community engagement, and policy development.

Based on the project's findings from working with local communities, successful implementation of protective practices that could reduce risks for disease transmission in at-risk individuals will require educational efforts to enhance individual understanding of disease risk factors.

The creation of the first interdisciplinary One Health platform in Jordan served as the starting point for future implementation of collaborative approaches to reducing emerging disease risk and as the foundation of a national One Health Committee.

FURTHER READING

- Ali, Mohamed, et al. "Cross-sectional surveillance of Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels and other mammals in Egypt, August 2015 to January 2016." *Euro Surveillance* 22.11 (2017): 30487. DOI: 10.2807/1560-7917.ES.2017.22.11.30487
- Kandeil, Ahmed, et al. "Isolation and characterization of a distinct influenza A virus from Egyptian bats." *Journal of Virology* 93.2 (2019). DOI: 10.1128/JVI.01059-18

For more information view the interactive report at **p2.predict.global**

SPECIAL FEATURES

THE FIRST NATIONAL COMMITTEE FOR ONE HEALTH IN THE MIDDLE EAST

In Jordan, the PREDICT team was instrumental in the development of a national One Health platform. Through multisectoral engagement including representation from a variety of relevant institutions along with outreach and partnership building, the PREDICT project established points of contact within the Ministry of Agriculture (MOA), Ministry of Health (MOH), Ministry of Environment (MOE), USAID/Jordan, World Health Organization (WHO), Food and Agriculture Organization (FAO), World Health Organization (OIE) and Royal Scientific Society (RSS) to serve on the first interdisciplinary One Health committee. This committee met regularly and served as a platform for future implementation of One Health approaches in-country. This collaboration is novel and serves as the first national committee for One Health in the area. Inspired by the PREDICT project's achievements, the committee is currently focused on progress, legacy, and the future of One Health in the country as the project has transferred leadership to the Jordanian Ministry of Health to lead future endeavors.

SPECIAL FEATURES

CHILDREN'S MUSEUM OUTBREAK EXHIBIT

World Health Day was celebrated on April 4 and 5, 2019. On this occasion, The Children's Museum, Jordan (CMJ) opened "Outbreak: Epidemics in a Connected World", an exhibition that ran from April 7 to December 2019. The aim of the exhibit was to promote the One Health message and communicate the importance of interdisciplinary responses to stop outbreaks. The messaging also centered around the impact outbreaks have on communities. The Museum partnered with the PREDICT team in Jordan, which provided expertise and consultations to develop locally relevant content, specifically related to JUST's work on new emerging infectious diseases that could become a threat to human health. Our team was actively engaged with children and their families and was tasked with communicating the role of wild animals in transmitting diseases to human populations. An animated short on disease outbreaks was also developed for the CMJ exhibit and featured the PREDICT project's One Health surveillance activities.

PREDICT
ETHIOPIA

ONE HEALTH IN ACTION (2015-2020)

Promoting vigilance for viral spillover from wildlife to people

Identified as part of a priority region for the emergence of new viral threats such as the Middle East respiratory syndrome coronavirus (MERS-CoV), a pathogen with origins in bats transmitted to people by camels, PREDICT Ethiopia forged strategic One Health partnerships in 2015 between UC Davis, the Aklilu Lemma Institute of Pathobiology at Addis Ababa University, and the Ethiopian Public Health Institute. PREDICT is one of the first initiatives in Ethiopia that takes a One Health approach to address wildlife zoonoses that pose substantial threats to human, animal and economic health such as MERS coronavirus. Through consultation with partners including the Ministry of Health and the Ethiopian Wildlife Conservation Authority, One Health surveillance sites were identified in high-risk interfaces for viral spillover and spread including areas in and around Awash National Park and Bati camel holding ground. Community engagement throughout the length of the project was paramount to ensuring the success and safety of our field teams, especially given the civil unrest and political instability in the country that affected field activities.

PREDICT put One Health into action by introducing innovative disease surveillance methods, strengthening laboratory and workforce capability, and improving capacity for surveillance and detection of zoonotic diseases and emerging viral threats. Working in support of the Global Health Security Agenda in Ethiopia, PREDICT's overall goals were to identify animal reservoirs and amplification hosts for zoonotic viruses, enhance real-time disease surveillance and work force capacity, and participate in collaborative One Health platforms that reduce the risk of disease spillover, amplification, and spread. Wildlife and human samples were screened for five priority virus groups suspected to be sources for new potential zoonotic pathogens impacting people. Through analysis of project data and findings, PREDICT was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

PREDICT strengthened two molecular diagnostic laboratories in country, both of which are now capable of detecting nationally recognized priority zoonoses and new and emerging viral disease threats, and which contribute to training and empowering a One Health workforce for sustained disease prevention, detection, and response. An example of regional capacity building at its finest, our partners in Ethiopia benefitted from knowledge and technical skill exchange with PREDICT teams in Tanzania and Uganda; an enduring legacy as these relationships and professional networks continue long after the project has ceased.

LOCAL PARTNERS

- Addis Ababa University, Aklilu Lemma Institute of Pathobiology (AAU, ALIPB)
- Ethiopian Public Health Institute (EPHI)
- Ministry of Health
- Awash Health Center
- Bati Health Center
- Ministry of Culture & Tourism
- Ethiopian Wildlife Conservation Authority
- Ministry of Livestock Development and Fisheries (MoLDF)
- National Animal Health Diagnostics and Investigation Center (NAHDIC)
- Food and Agricultural Organization (FAO)

Global Health Security Agenda

30

DEVELOPED the One Health Workforce by training 30 people in Ethiopia.

>1.3K

OPERATIONALIZED One Health surveillance and sampled over 1.3K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Addis Ababa University, Aklilu Lemma Institute of Pathobiology

2,828
TESTS

3 PREDICT **8** KNOWN

DETECTED 11 unique viruses in both animal and human populations.

DESALEGN BELAY

Human Surveillance Lead, *Ethiopian Public Health Institute*

“Prevention starts with detection, and PREDICT has brought new lab capacity and a One Health surveillance approach to prevent outbreaks.”

YOHANNES NEGASH

Wildlife Surveillance Laboratory Lead, *Addis Ababa University, Aklilu Lemma Institute of Pathobiology*

“PREDICT is one of the best coordinated projects I’ve ever seen. I have learned a lot of techniques from field sampling to laboratory testing. We have learned how to organize tasks and how to collaborate with others, and how to manage a whole project. PREDICT has taught me to follow up and harmonize all activities and to share an approach across countries. PREDICT can detect emerging and known zoonotic viruses which is very important for early warning and detection of potential pandemics.”

ACHIEVEMENTS

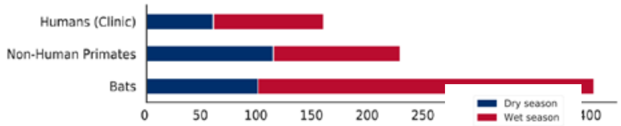
- First known project to utilize a One Health approach for zoonotic disease surveillance in bats in Ethiopia
- Implemented new field methods for wildlife disease surveillance
- Created new collaborations between universities, institutions, sectors, and countries
- Established conventional PCR laboratory techniques in two laboratories in country
- Participated in the establishment of the Ethiopian National One Health Steering Committee made of representatives from EPHI, Ministry of Agriculture, Ministry of Forestry, Environment and Climate Change, four universities, EWCA, Ministry of Higher Education, and included partners from the WHO, CDC, and FAO
- Strengthened regional networks and communication channels across East Africa
- Identified a new alphacoronavirus – PREDICT CoV-114 in 2 bat species in Ethiopia

ONE HEALTH SURVEILLANCE

Concurrent sampling locations in Ethiopia were strategically chosen to reflect high-risk animal-human interfaces for viral spillover and spread. These included: areas in and adjacent to Awash National Park, in the towns of Awash and Metehara, Afar and Oromia regions; and Bati town, Amhara region, close to the Mile Serdo Wildlife Reserve. Both of these areas include growing urban centers, commercial and subsistence farms, livestock markets, and wildlife reserves. In both Awash-Metehara and Bati, wildlife sampling areas reflected a gradient of rural to urban human density impact. Syndromic (febrile) patients were enrolled at local health centers, located in the urban city center.

From 2016-2018, the PREDICT team safely collected and tested samples from 589 bats and 247 non-human primates during 23 sampling events over both the dry and rainy seasons. From May-October 2018, 310 febrile humans were longitudinally enrolled, sampled at the Awash and Bati Health Centers. Specimens collected from both wildlife and people included oral and nasal swabs, saliva, feces, urine, whole blood and serum.

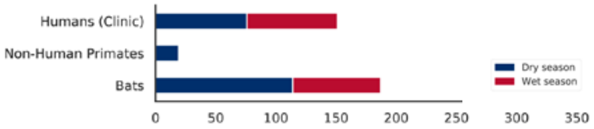
AWASH-METEHARA



Numbers of individuals sampled by taxa group in Awash-Metehara

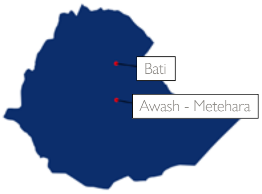
Bats were safely and humanely sampled in a roadside cave and commercial and domestic buildings in Metehara town. Samples from bats in the roadside cave were collected from a 10mx15m volcanic blister cave adjacent to the main highway running between Addis Ababa and Djibouti. Interestingly, there is a carwash service adjacent to the cave that uses water collected from the cave floor to wash large lorries overnighting along the highway. Young men also often sleep in this cave. Bats were also sampled from a large commercial store building and a villager's residence. Non-human primates were sampled in areas in and around Awash National Park. Febrile patients were enrolled for sampling and completion of a behavioral risk questionnaire at the Awash Health Center.

BATI



Numbers of individuals sampled by taxa group in Bati

In Bati there is a camel holding ground, which is a regionally important trading point for camels moving through Ethiopia and the surrounding region. Bat sampling locations in this area included the local primary school, the Bati Health Center (where febrile patients were enrolled for sampling and behavioral risk questionnaires), and traditional styled domestic residences (hay roofed tukuls).



Awash-Metehara
= bats; humans;
non-human primates

Bati = bats; humans;
non-human primates

WILDLIFE SPECIES SAMPLED AND TESTED

	2016	2017	2018	TOTAL
AWASH-METEHARA BATS	93	109	200	402
LITTLE FREE-TAILED BAT (*Chaerephon pumilus*)	60	88	80	228
LESSER MOUSE TAILED BAT (*Rhinopoma hardwickii*)	33	21	120	174
BATI BATS	0	0	187	187
MIDAS FREE TAILED BAT (*Mops midas*)	0	0	180	180
SOMALI SEROTINE BAT (*Neoromicia cf. somalica*)	0	0	7	7
AWASH-METEHARA NON-HUMAN PRIMATES	7	21	200	228
GRIVET MONKEY (*Chlorocebus aethiops*)	1	4	12	17
OLIVE BABOON (*Papio anubis*)	1	10	40	51
SACRED BABOON (*Papio hamadryas*)	5	7	148	160
BATI NON-HUMAN PRIMATES	0	0	19	19
GRIVET MONKEY (*Chlorocebus aethiops*)	0	0	19	19

VIRUS DETECTION

PREDICT's strategy for virus detection included screening samples using broadly reactive consensus PCR (cPCR) for five priority virus groups: coronaviruses, filoviruses, flaviviruses, paramyxoviruses, and influenza viruses. Viruses detected via these assays were sequenced to investigate their relationship to known pathogens and samples were prioritized for further characterization based on these results. This approach allows for detection of both known and new viruses and improves our understanding of the potential for the virus to cause disease in humans and/or animals.

Wildlife samples were safely tested at the Addis Ababa University (AAU)/Aklilu Lemma Institute of Pathobiology (ALIPB) beginning in 2017. Specimens from patients collected at the Awash and Bati Health Centers were safely tested at the Ethiopia Public Health Institute (EPHI) laboratory beginning in 2018. Mentoring and remote support from regional and global laboratory staff helped both laboratories successfully test samples, trouble shoot problems, and ensure test reliability. Quality assurance and control was closely monitored, with some specimens tested in parallel at UC Davis to ensure new laboratory test reliability. cPCR positive samples were sent to PREDICT's global reference laboratory at UC Davis for confirmatory testing and sequencing.

(Left-right) Carwash service using water collected from the cave where bats were sampled in Awash-Metehara; primary school where bats were sampled in Bati.

PHOTOS: PREDICT/ETHIOPIA

VIRUS FINDINGS IN PEOPLE

Of the 310 humans tested, we detected and confirmed viruses in 27 patients, including: influenza A (n=21), influenza B (n=2), betacoronavirus 1-OC43 (n=2), measles virus (n=1), and human parainfluenzavirus (n=1). More viruses were detected in people in early May and July (rainy season). EPHI partners may be pursuing further subtyping of influenza viruses.

VIRUS FINDINGS IN WILDLIFE

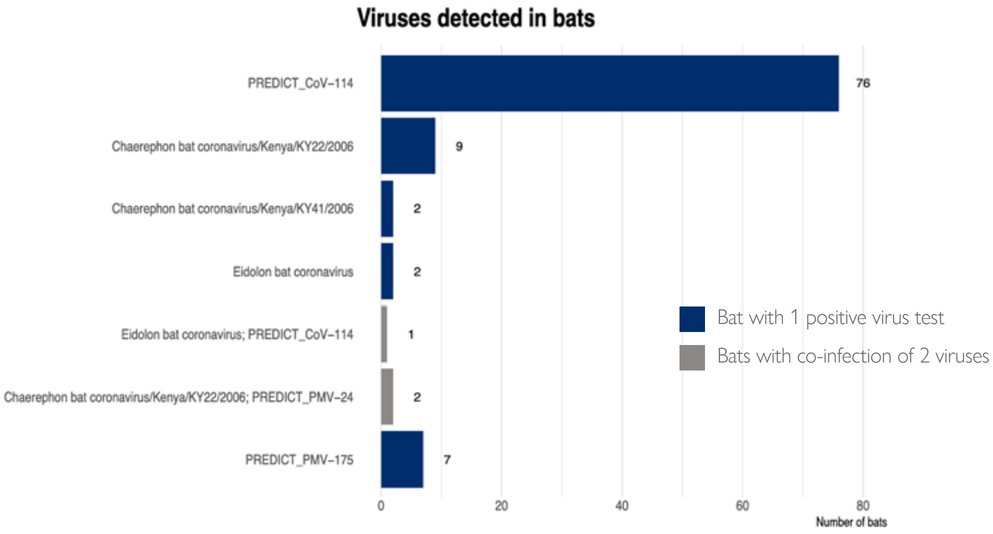
Awash-Metehara bat species sampled included Little free-tailed bat (*Chaerephon pumilus*) and Lesser mouse-tailed bats (*Rhinopoma hardwickii*); Bati bat species sampled included Midas free-tailed bats (*Mops midas*) and Somali serotine bats (*Neoromicia cf. somalica*). Out of 589 bats tested, we identified six unique viruses in 99 bats, including three known coronaviruses, two new paramyxoviruses, PREDICT_PMV-24 and PREDICT_PMV-175, and one novel coronavirus, PREDICT_CoV-114. PREDICT_CoV-114 virus is a new coronavirus found in bats belonging to the *Alphacoronavirus* genus. Three bats exhibited viral co-infections; two bats tested positive for both Chaerephon bat coronavirus/Kenya/KY22/2006 and PREDICT_PMV-24, and one bat tested positive for PREDICT_CoV-114 and Eidolon bat coronavirus. No viruses were detected in the non-human primate samples.

Bats were more likely to be positive for any virus if sampled at the Awash-Metehara site (21%) vs. Bati site (7%). Additionally, a higher proportion of subadult age class bats were positive for any virus than adult bats of either sex, consistent across both sites. Across all sites, more viruses were detected during the rainy season (late June to September).

We detected the novel PREDICT_CoV-114 virus at both sites and during multiple years of sampling, with the majority of positive cases detected in August. In Awash-Metehara, this virus was detected in 72 Lesser mouse-tailed bats, during sampling trips conducted in 2016 and 2018. In Bati, this virus was found in five Midas free-tailed bats during 2018 sampling trips (only one year of sampling occurred at Bati). We detected PREDICT_CoV-114 virus in 41.4% of all Lesser mouse-tailed bats sampled. Subadult bats of both sexes were more likely to be positive for this virus than adult bats. Rectal swabs (n=49) were the most common specimen type to yield a virus sequence, though sequences were confirmed in both rectal and oral swabs collected from 23 bats, and from oral swabs collected from an additional five bats.

Our findings parallel those of a global investigation of the diversity of coronaviruses in bats (Anthony et al., 2017), with subadult bats more likely to test positive than adults, and feces/rectal swabs most likely to yield a coronavirus positive finding. However, our detection of the PREDICT_CoV-114 virus in samples collected almost exclusively during the rain season diverges from previously published results, and the lack of samples testing positive for this virus in 2017 at the Awash-Metehara site raises further questions about the epidemiology and transmission dynamics of PREDICT_CoV-114 as well as the population ecology and life history of Lesser mouse-tailed bats in Ethiopia.

There is no evidence at this time to suggest any of the viruses detected in Ethiopian wildlife pose a threat to human health. However given the recent emergence of novel betacoronavirus SARS-CoV-2, additional investigation into the ecology, evolution and global distribution of coronaviruses in wildlife is warranted. Further analysis of the alphacoronavirus PREDICT_CoV-114 identified in Ethiopia is ongoing at partner laboratories at UC Davis and Columbia University.



VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Awash Health Center	2	1	1
	PREDICT_CoV-114	Lesser Mouse-Tailed Bat, Midas Free-Tailed Bat	Awash-Metehara, Bati	77	76	1
	Chaerephon bat coronavirus/Kenya/KY22/2006	Little Free-Tailed Bat	Awash-Metehara	11	4	7
	Chaerephon bat coronavirus/Kenya/KY41/2006	Little Free-Tailed Bat	Awash-Metehara	2	0	2
	Eidolon bat coronavirus	Lesser Mouse-Tailed Bat, Little Free-Tailed Bat, Somali Serotine	Awash-Metehara, Bati	3	1	2
Paramyxovirus	Human parainfluenzavirus 3	Human	Bati Health Center	1	1	0
	Measles virus	Human	Bati Health Center	1	1	0
	PREDICT_PMV-24	Little Free-Tailed Bat	Awash	2	0	2
	PREDICT_PMV-175	Midas Free-Tailed Bat	Bati	7	4	3
Influenza virus	Influenza A	Human	Awash Health Center, Bati Health Center	21	7	14
	Influenza B	Human	Awash Health Center	2	0	2
Total				**129**	**95**	**34**

PARTICIPANT CHARACTERISTICS ACROSS ALL SURVEILLANCE SITES

SITES	INFLUENZA A (n=21)	INFLUENZA B (n=2)	BETACORONAVIRUS 1 (OC43) (n=2)	MEASLES VIRUS (n=1)	HUMAN PARAINFLUENZAVIRUS 3 (n=1)	NEGATIVE (n=283)	OVERALL (n=310)
SEASON							
DRY	14 (66.7%)	2 (100%)	1 (50%)	0 (0%)	0 (0%)	119 (42.%)	136 (43.9%)
WET	7 (33.3%)	0 (0%)	1 (50%)	1 (100%)	1 (100%)	164 (58%)	174 (56.1%)
GENDER							
FEMALE	11 (52.4%)	1 (50%)	1 (50%)	1 (100%)	0 (0%)	178 (62.9%)	192 (61.9%)
MALE	10 (47.6%)	1 (50%)	1 (50%)	0 (0%)	1 (100%)	105 (37.1%)	118 (38.1%)
AGE							
CHILD (<18)	7 (33.3%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	56 (19.8%)	64 (20.6%)
ADULT (≥18)	14 (66.7%)	2 (100%)	2 (100%)	1 (100%)	0 (0%)	227 (80.2%)	246 (79.4%)
HEALTH CENTER							
AWASH	12 (57.1%)	2 (100%)	2 (100%)	0 (0%)	0 (0%)	143 (50.5%)	159 (51.3%)
BATI	9 (42.9%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	140 (49.5%)	151 (48.7%)

EPIDEMIOLOGICAL & BEHAVIORAL RISK

HEALTH CENTER SURVEILLANCE

Inclusion criteria for enrollment at both the Bati or Awash Health centers included children older than two and adults exhibiting a fever of 38°C or greater and symptoms consistent with a fever of unknown origin, influenza-like illness, severe acute respiratory illness, acute encephalitis or hemorrhagic fever of unknown origin. A total of 310 patients were enrolled and tested, 159 from Awash and 151 from Bati. 93% of patients resided in the town where the health center was located. Highest education level completed by participants varied, with the majority of adult participants having no formal education (35%) or only completing primary school (44%); 13% of adult participants completed secondary school, and 8% of adults obtained a college or higher education degree. Of the 310 patients, 289 had fevers ≥38°C on admission, with an average fever of 38.4°C and approximately three days duration. The most common presenting symptoms at enrollment included: 1) fever, 2) headache, 3) chills, 4) joint pain, and 5) inappetence. Fever and headache were the most common co-presenting symptoms. Two patients from Awash HC and eight patients from Bati HC tested positive for *Plasmodium falciparum* (malaria) based on microscopic examination of a blood smear. These patients were included in testing using the PREDICT testing protocol.

SELF-REPORTED BELIEF OF CAUSE OF ILLNESS

Of those >12 years old surveyed, multiple responses were provided for the causes of illness over the past year. Across all education levels, the top five responses included: 1) bad food or water, 2) contact with sick people, 3) don't know, 4) contact with other animals, and 5) contact with wild animals. For those with no formal education, the top cause of illness reported was "don't know."

LIVELIHOODS OF SURVEYED INDIVIDUALS

Across both sites, women enrolled were most often homemakers or engaged in activities or businesses that did not involve livestock or domestic animals. Men were more likely to be students, engaged in farming and crop production, or unemployed.

SELF-REPORTED ANIMAL-HUMAN CONTACT IN THE PAST YEAR

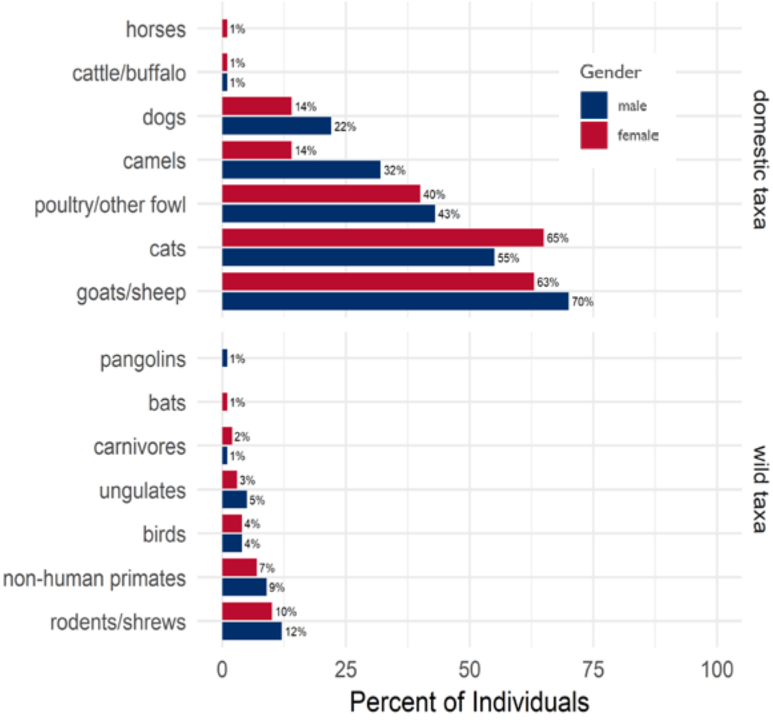
When asked about contact with animals and the type of contact experienced over the past year, rodents were the most common type of wildlife encountered by men and women, closely followed by non-human primates, a finding consistent across both sites. No males reported contact with bats, and only 1% of women reported bat contact, especially surprising because bats living in peri-domestic locations were sampled at both sites. Both genders reported small ruminants as the most common type of livestock encountered, with men more likely to have contact with camels.

Respondents also self-reported their behaviors following an animal scratching or biting them, or after experiencing a cut or injury when butchering or slaughtering an animal. The majority reported taking action either through visiting a doctor, bandaging or washing the wound, or letting someone else take over after such an event.

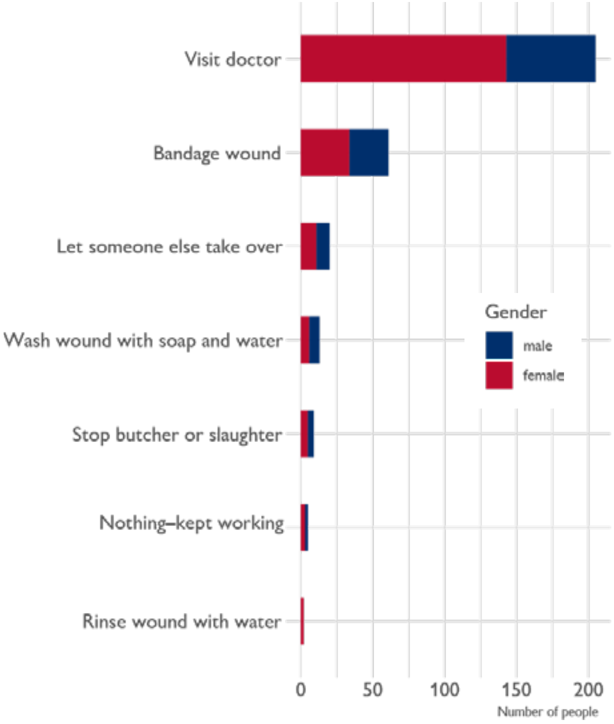
ENROLLED AND TESTED INDIVIDUALS BY AGE AND GENDER

HEALTH CENTER	AWASH (n=159)	BATI (n=151)	OVERALL (n=310)
GENDER			
FEMALE	101 (63.5%)	91 (60.3%)	192(61.9%)
MALE	58 (36.5%)	60 (39.7%)	118 (38.1%)
AGE			
ADULT (<18)	135 (84.9%)	111 (73.5%)	246 (79.4%)
CHILD (≥18)	24 (15.1%)	40 (26.5%)	64 (20.6%)
AGE (YEARS)			
MEDIAN [MIN, MAX]	30.0 [3,80]	25.0 [3,60]	27.5 [3,80]

SELF-REPORTED ANIMAL-HUMAN CONTACT IN THE LAST YEAR



ACTION WHEN SCRATCHED/BITTEN/CUT



(Left-right) Baboon eats a banana while also providing the team with non-invasively collected saliva sample; child and baboon share play space adjacent to Awash National Park

PHOTOS: PREDICT/ETHIOPIA

RISK REDUCTION & COMMUNICATION

Engagement of local officials, community members and other stakeholders in the Bati and Awash-Metehara areas was a core component of project activities. Through regular meetings with community officials, our team introduced the One Health approach, and included local leaders in all steps of the project. This support was critical for obtaining local permissions, ensuring staff safety when performing sampling activities, and willingness to participate in the human surveillance portion. In addition, it was critical for gaining trust and provided a platform for sharing project findings, insights and recommendations, including successes and breakthroughs from PREDICT teams in Africa and around the world.

In Ethiopia, our team raised awareness about risk factors for zoonotic diseases in people from contact with wildlife; demographics, livelihood, and wildlife contact are all potential risk factors that could be associated with exposure to new and previously detected viruses in wildlife. We worked with communities, health centers, government officials and other stakeholders to share findings and recommend potential solutions. The high-risk interfaces we targeted in Ethiopia allowed our teams to gather information regarding community perceptions about living in close proximity to wildlife and livestock, as well as their understanding of the risks animals pose to health. While most people did not report contact with bats, our wildlife surveillance team was frequently asked to remove bats they captured for sampling, providing the opportunity to discuss the importance of bats to the ecosystem along with ways to reduce risks and live safely with them. There remains a need and opportunity for additional education on the health and conservation elements of living in close proximity to wildlife, particularly bats, in these areas, an initiative our PREDICT team kickstarted by distributing Amharic editions of PREDICT's behavior change and risk reduction resource *Living Safely with Bats* to government and health officials for use in community outreach and education events.

Cover of Amharic translation of Living Safely with Bats

Brett Smith of UC Davis PREDICT/Global with laboratory team trained at AAU/ALIPB

PHOTOS: PREDICT/ETHIOPIA

STRENGTHENING CAPACITY

Our team in Ethiopia greatly benefited from the knowledge and skills of other PREDICT countries in the East Africa region, including the PREDICT teams in Uganda and Tanzania, and are the global reference labs. This regional exchange enabled the exchange of knowledge and technical skills while contributing to improved cross border collaboration and professional networking. The Ethiopia team, including professionals and faculty from ALIPB/AAU, NAHDIC and EPHI were trained on disease detection techniques and the One Health approach to disease surveillance. In addition, staff at the Awash and Bati Health Centers all received training on safe and ethical sample collection and storage and in conducting behavioral risk interviews. Addis Ababa University staff and PhD students were trained in laboratory techniques including biosafety and biosecurity, safe and humane wildlife surveillance, and safe and biosecure sample collection and storage. Early on in the project, core members from the Ethiopia team traveled to sites in Uganda and Tanzania for in-depth training on wildlife surveillance and virus detection. Brett Smith from UC Davis led laboratory trainings at AAU/ALIPB and supported the establishment of a molecular diagnostic laboratory enabling the detection in country of both known and new viral threats. Additionally, Dr. Grace Mwangoka, Human Surveillance lead for PREDICT Tanzania spent several weeks in Ethiopia supporting the training and launch of our human surveillance teams at the Awash and Bati Health Centers.

(Left-right) Dr. Grace Mwangoka, Human Surveillance Lead for PREDICT/Tanzania, leads a training for Ethiopian Public Health Institute staff members, Desalegn Belay, Adamu Tayachew and Mesfin Menghesha; Yohannes Negash of AAU/ALIPB training EPHI and AAU staff on cPCR techniques.

PHOTOS: PREDICT/ETHIOPIA

NATIONAL ONE HEALTH STEERING COMMITTEE

The One Health approach is particularly useful to bring diverse stakeholders to work together to prevent, detect and and respond to increasing concerns posed by emerging public health threats. In October 2018, Ethiopia established a National One Health Steering Committee (NOHSC), improving multi-sectoral collaboration to ensure optimal health for people, animals, and the ecosystem. PREDICT Ethiopia collaborated with partners from USAID/ Preparedness and Response, USAID/One Health Central and Eastern Africa (OHCEA), the Food and Agriculture Organization (FAO), CDC, Global One Health Initiative and four Ethiopian government ministries to launch a National One Health Council. The Ministry of Health (MOH), Ministry of Agriculture and Livestock Resources (MoALR), Ministry of Environment, Forest and Climate Change (MEFCC), and Ministry of Culture and Tourism (MOCT), signed a One Health Memorandum of Understanding with the United States Agency for International Development (USAID). PREDICT also contributed to the development of a five-year national One Health Strategic Plan that enables Ethiopia to reduce the risk of disease outbreaks and to prevent, control and respond to known and emerging infectious diseases. A National One Health Steering Committee composed of senior officials from the ministries is already in place to institutionalize and implement the plan by 2022. These successes exemplify the contributions of the PREDICT team to national Global Health Security Agenda objectives, a lasting legacy for success in disease prevention, control, and response.

PRACTICAL IMPLICATIONS

- Two laboratories, both part of the national laboratory system and representing animal and human health sectors are now empowered with the capacity and knowledge to detect known and newly emerging viral threats.
- PREDICT set a new precedent, putting One Health in action and supporting the creation of the National One Health Steering Committee and the One Health Strategic plan. These initiatives affirm the commitment of the Government of Ethiopia and national stakeholders to continue One Health work in country.
- Awash and Bati Health Clinic staff are more aware of zoonotic diseases and the importance of asking about risk factors for transmission, such as history of animal contact.
- Disease surveillance in wildlife populations, particularly in bats, is an emerging frontier in Ethiopia. Our detection of the PREDICT_CoV-114 virus in Lesser Mouse Tailed bats and Midas free tailed bats is the first published findings on a new virus in bats sampled in Ethiopia.
- Additional zoonotic disease surveillance and population ecology studies of wildlife, particularly bats, in Ethiopia will be an important component of One Health surveillance work in the future.

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- *Living Safely with Bats* Amharic edition, available at: https://p2.predict.global/living-safely-with-bats-book

PREDICT

GHANA

ONE HEALTH IN ACTION (2014-2020)

Using a One Health approach to enhance national health security by strengthening disease surveillance and laboratory capacities for detection, response, and prevention of current and emerging zoonotic viral threats.

In Ghana, a rapidly growing economy and population have led to deforestation, agricultural expansion, human migration, and vibrant regional trade – all factors associated with risk for disease emergence and spread (Daszak et al., 2001). PREDICT was initiated in Ghana in 2016 to enhance existing capacities to conduct surveillance for emerging viral zoonoses of pandemic potential (filoviruses, coronaviruses, paramyxoviruses, flaviviruses, arenaviruses (humans only), and influenza viruses) at high-risk disease transmission interfaces between wildlife, livestock, and people.

PREDICT used a One Health approach to conduct disease surveillance in bat, rodent, primate, and human populations in a rural setting near the Boabeng-Fiema Monkey Sanctuary and in a highly urban setting in the capital city. In the rural communities of the Nkoranza North District, people depend heavily on agriculture for their livelihoods and come into close contact with bats and rodents in their homes and while working and hunting in their agricultural fields. Traditional beliefs and local law forbid physical harm to the monkeys in the sanctuary, which come into the village daily to enter peoples' homes, raid food stores, and feed alongside livestock in the agricultural fields. Tourists visiting the sanctuary also feed the monkeys, a practice which increases the populations' dependence on artificial food and is thought to contribute to population growth, leading to human-primate conflicts and heightened risk of shared diseases.

PREDICT also targeted for surveillance, one of the largest known urban bat roosts in West Africa, an *Eidolon helvum* colony in Accra's city center. Recent studies in Ghana suggest that *E. helvum* harbor zoonotic or potentially zoonotic viruses, including henipaviruses (Drexler et al., 2012; Hayman et al., 2008), lyssaviruses (Hayman et al., 2008b; Suu-ire et al., 2017), and ebola viruses (Hayman et al., 2010). This is notable because *E. helvum* migrate long distances, roost in high densities in large colonies across Africa, and are hunted for consumption (Kamins et al., 2011).

In addition to sampling people in the high-risk communities, PREDICT conducted syndromic surveillance in patients presenting with acute febrile illness of unknown origin to clinics, including a large referral hospital in Accra's city center, that receives cases for most medical disasters and disease outbreaks in the country. The clinics served the communities where PREDICT conducted surveillance.

Through analysis of project data and findings, PREDICT was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats. When requested by the government of Ghana, PREDICT also utilized a One Health approach to assist with disease outbreak investigations. In addition, the team played a key role in activities under the Global Health Security Agenda, including evaluation of the country's strengths, gaps, and priority actions for enhancing national health security; prioritization of zoonoses of public health concern in Ghana; and development of a national One Health policy.

IMPLEMENTING PARTNERS

- 37 Military Hospital
- Ghana Health Service
- Noguchi Memorial Institute for Medical Research, University of Ghana
- United States Agency for International Development
- University of California, Davis
- Veterinary Services Department, Ministry of Food and Agriculture
- Wildlife Division of the Forestry Commission, Ministry of Land and Natural Resources

OTHER PARTNERS

- Ghana Armed Forces
- Food and Agriculture Organization of the United Nations
- Ministry of Health Emergency Operations Center
- Ministry of the Interior National Disaster Management Organization
- NAMRU-3
- National Public Health Reference Laboratory
- Partnership for Health Care Improvement
- United States Centers for Disease Control & Prevention
- World Health Organization

>60

DEVELOPED the One Health Workforce by training more than 60 people in Ghana.

>3.4K

OPERATIONALIZED One Health surveillance and sampled over 3.4K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Accra Veterinary Laboratory
- Noguchi Memorial Institute for Medical Research, University of Ghana

20,480
TESTS

6 PREDICT

10 KNOWN

DETECTED 16 unique viruses in both animal and human populations.

COL SAMUEL BEL-NONO

Country Coordinator, *Retired from the Ghana Armed Forces Medical Services*

"PREDICT is a versatile project that brings together disease surveillance and diagnostic laboratory networks to support the Global Health Security Agenda and One Health platform in Ghana. The project has expanded the tools and approaches to viral disease investigations and strengthened national capacities for prevention, detection, and response to zoonotic viruses."

EVANGELINE OBODAI

Human Laboratory Lead, *Noguchi Memorial Institute for Medical Research, University of Ghana*

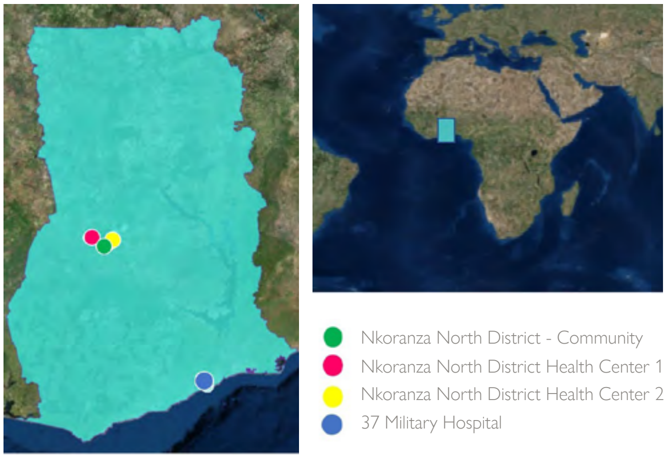
"PREDICT is directly encouraging inter-ministerial collaboration and providing the opportunity for partners to work together in the field and laboratories; directly strengthening cross-sectoral information and data sharing; and helping to enhance capabilities for early zoonotic disease detection, prevention, and response. PREDICT has been able to strengthen laboratory capacity for zoonotic disease detection in Ghana by providing a useful basic PCR tool/platform that comes in handy for screening for novel viruses."

ACHIEVEMENTS

- Expanded the scope of national disease surveillance activities in Ghana to include a focus on zoonotic viruses of major public health significance
- Strengthened national capacities to detect novel viruses that may be the cause of undiagnosed illnesses in Ghana; the Virology Department at NMIMR conducts diagnostic testing for viral pathogens in clinical cases, for the Ministry of Health/Ghana Health Service; PREDICT virus detection protocols for filoviruses, arenaviruses, flaviviruses, influenza viruses, coronaviruses, and paramyxoviruses are now included in the molecular diagnostic suite used to investigate the cause of illness for these referred cases
- Enhanced capacity for the Ghana Health Service vector surveillance system by incorporating PREDICT's protocol for safe specimen collection into the field activities and PREDICT's virus detection protocols into the suite for molecular-based assays used to screen rodent and mosquito samples for evidence of viruses of public health importance, including Ebola, Marburg, Crimean-Congo hemorrhagic fever, dengue, Zika, chikungunya, yellow fever, Lassa, and other arenaviruses
- PREDICT Ghana's two human syndromic surveillance sites in the Nkoranza North District of the Bono East Region were recommended for designation as sentinel sites for zoonotic disease surveillance by the Head, Disease Surveillance Department, Ghana Health Service
- Enhanced national health security by playing a key role in the Joint External Evaluation of IHR core capacities, Zoonotic Diseases Prioritization Workshop, and development of the implementation plan for the Global Health Security Agenda Zoonoses Action package for Ghana
- Strengthened institutional collaboration for implementation of One Health approaches through increasing awareness of One Health at the ministerial level and contributions to development of the national One Health policy
- Implemented One Health approach to support the Ghana government in investigation of a suspected Lassa fever case

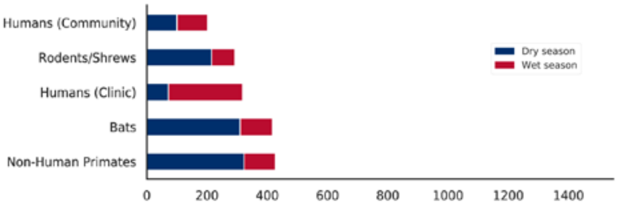
ONE HEALTH SURVEILLANCE

PREDICT's One Health surveillance approach was designed to strengthen capacity for detection of emerging viral threats and to improve our understanding of risk of zoonotic diseases in communities with close and frequent animal contact. Our team safely and humanely sampled wildlife (bats, rodents, and non-human primates) and people concurrently, targeting high-risk populations for viral spillover, amplification, and spread in rural and highly urbanized settings in Ghana. We also conducted syndromic surveillance of patients presenting to local clinics serving the communities at our sites. Data collection and sampling was performed longitudinally over a three-year period during both the rainy and dry seasons. Species identification of bats and rodents was confirmed using DNA barcoding, a molecular-based laboratory assay.



PREDICT surveillance sites in Ghana

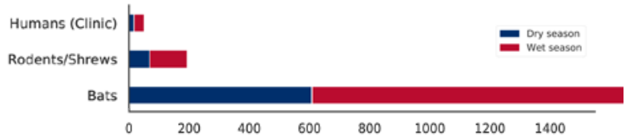
NKORANZA NORTH DISTRICT



Numbers of individuals sampled by taxa group in Nkoranza North District

Our team conducted surveillance in communities within the Nkoranza North District near the Boabeng-Fiema Monkey Sanctuary. These community members are primarily farmers who rely on extensive small-scale crop and livestock production (primarily domestic fowl, sheep, and pigs) and to a lesser degree hunting for their livelihoods. The Boabeng-Fiema Monkey Sanctuary, which draws visitors from around the world, is home to over 700 monkeys (Black and White Colobus and Lowe's Mona monkey) that interact with the communities through daily visits to the villages where they enter homes and raid food stores. The monkeys also have contact with sanctuary visitors who feed them by hand, a practice discouraged by local officials. Interspersed among the patches of sanctuary forest are cashew and mango orchards, which provide foraging habitat for a diversity of fruit bats, bringing them into close proximity to these farming communities. In addition to sampling wildlife (bats, rodents, non-human primates) and people at the villages, we conducted syndromic surveillance at two nearby health centers where we targeted patients presenting with acute febrile illness.

ACCRA



Numbers of individuals sampled by taxa group in Accra

Our team also conducted surveillance on the grounds of the 37 Military Hospital in Accra's bustling city center, which is home to one of the largest known urban fruit bat (*Eidolon helvum*) colonies in West Africa (~250,000 bats). The bats migrate long distances, congregating in their urban roosts during the dry season and migrating into the northern savannas at the onset of the wet season. The bats roost and fly over a busy transit center where people gather for public transportation. In addition to sampling wildlife at this site, we targeted patients presenting to the 37 Military Hospital, a large referral hospital that receives patients from the local community and from across the country, especially during disease outbreaks.

VIRUS DETECTION

PREDICT's strategy for virus detection in Ghana included screening samples using broadly reactive consensus PCR (cPCR) for priority virus groups: coronaviruses, filoviruses, flaviviruses, paramyxoviruses, influenza viruses, and arenaviruses (humans only). Viruses detected via these assays were sequenced to investigate their relationship to known pathogens and samples were prioritized for further characterization via sequencing based on these results. This approach allows for detection of both known and novel viruses and improves our understanding of the potential for the virus to cause disease in humans and/or animals.

Using this strategy, our teams safely tested samples from 565 people and 1,620 wild animals. We identified both known and new viruses in bats and rodents and detected known viral pathogens circulating among people that were not monitored through national surveillance programs (see Virus Findings Table). In patients with acute febrile illness, we identified common causes of illness, including influenza A (22 cases), influenza B (five cases), betacoronavirus 1 (OC 43) (four cases), human parainfluenzavirus 2 and 3 (one case each), and mumps (one case). Influenza A virus (two cases) and human coronavirus 229E (one case) were also detected in individuals through the community-based surveillance. These findings shed light on viral causes of respiratory illness, which is common in the community and an important cause of mortality in the country.

In wildlife, we identified three new paramyxoviruses in rodents and shrews and two paramyxoviruses in bats. We detected several coronaviruses in bats, including a new Betacoronavirus in a Gambian epauletted fruit bat (*Epomorphorus gambianus*).

Most commonly, the Betacoronaviruses, Kenya bat coronavirus/BtKY56/BtKY55 and Eidolon bat coronavirus, were detected (81 and 77 bats, respectively). Four bats also tested positive for the Alphacoronavirus Chaerephon bat coronavirus/Kenya/KY22/2006.

Among species of bats with detections of Kenya bat coronavirus/BtKY56/BtKY55, the percentage of positive bats was highest in *Epomops buettikoferi* (33%) followed by *Mops condylurus* (16%), *Epomophorus gambianus* (15%), *Epomops franqueti* (6%), and *Eidolon helvum* (2%). The virus was detected most commonly at the Nkoranza North District site with bats sampled at this site eight times more likely to test positive for this virus as compared to the 37 Military Hospital site. In addition, bats were five times more likely to test positive for the virus during the rainy season as compared to the dry season.

Eidolon bat coronavirus was most commonly detected in *Eidolon helvum* bats (33% positive), followed by Gambian Epauletted fruit bats (1% were positive). The virus was primarily detected at the 37 Military Hospital site where we sampled bats at the large urban roost. (See the special feature below for a more in-depth investigation of the dynamics of coronavirus shedding in urban *Eidolon helvum* bats in Ghana). None of the non-human primate samples tested positive for the target viruses.

These findings contribute to our knowledge base on viruses circulating among wild animals at these high-risk interfaces. For instance, our findings suggest that coronaviruses circulate among bats more commonly during the rainy season when the dams are weaning pups and there are higher numbers of susceptible individuals in the population. Detection of these viruses in wildlife provides opportunities to learn more about their ecology and risk of cross-species transmission and to identify mitigation strategies aimed at preventing human infections.

NMIMR has incorporated the PREDICT virus detection protocols into its diagnostic platforms for screening samples from clinical cases with illnesses of unknown etiology

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Nkoranza North District Health Centers 1 & 2, 37 Military Hospital	4	2	2
	Coronavirus 229E (Human strain)	Human	Nkoranza North District	1	1	0
	PREDICT_CoV-102	Gambian Epauletted Fruit Bat	Nkoranza North District	1	0	1
	Chaerephon bat coronavirus/Kenya/KY22/2006	Angolan Free-Tailed Bat, Straw-Coloured Fruit Bat, Gambian Epauletted Fruit Bat	Nkoranza North District, 37 Military Hospital Grounds	4	3	1
	Eidolon bat coronavirus	Gambian Epauletted Fruit Bat, Straw-Coloured Fruit Bat	Nkoranza North District, 37 Military Hospital Grounds	77	52	25
	Kenya bat coronavirus/BtKY56/BtKY55	Angolan Free-Tailed Bat, Buettikofer's Epauletted Fruit Bat, Franquet's Epauletted Fruit Bat, Gambian Epauletted Fruit Bat, Straw-Coloured Fruit Bat	Nkoranza North District, 37 Military Hospital Grounds	81	51	30
Paramyxovirus	Human parainfluenzavirus 2	Human	Nkoranza North District Health Center 1	1	1	0
	Human parainfluenzavirus 3	Human	Nkoranza North District Health Center 1	1	0	1
	Mumps virus	Human	Nkoranza North District Health Center 2	1	0	1
	PREDICT_PMV-15	Angolan Free-Tailed Bat	Nkoranza North District	1	0	1
	PREDICT_PMV-56	Straw-Coloured Fruit Bat	37 Military Hospital Grounds	2	2	0
	PREDICT_PMV-168	African Giant Shrew	37 Military Hospital Grounds	3	2	1
	PREDICT_PMV-170	African Giant Shrew	37 Military Hospital Grounds	1	0	1
	PREDICT_PMV-171	Deroo's Mouse	Nkoranza North District, 37 Military Hospital Grounds	7	5	2
Influenza virus	Influenza A	Human	Nkoranza North District, Nkoranza North District Health Centers 1 & 2, 37 Military Hospital	24	19	5
	Influenza B	Human	Nkoranza North District Health Centers 1 & 2	5	0	5
Total				**214**	**138**	**76**

EPIDEMIOLOGICAL & BEHAVIORAL RISK

Our PREDICT team partnered with local communities to learn more about people's awareness and perceptions of risk of zoonotic diseases and the environmental factors and types of activities they engage in that might influence the risk of disease transmission, including human demographics, livelihood activities, animal distributions, animal contact, and food safety and sanitation practices. From November 2017 to December 2019, 653 individuals participated in the syndromic and community-based surveillance efforts (264 community members and 342 patients at the Nkoranza North District site and 47 patients at the 37 Military Hospital (see Participant characteristics table below)). Participants were enrolled across the rainy and dry seasons. Each person was sampled and asked to fill a questionnaire that gathered information on potential risk factors for zoonotic viral spillover and spread.

We conducted surveillance at local clinics in the Nkoranza North District communities (NNDHCs) and the 37 Military Hospital (37MH) to better understand viral causes of illness among our target communities. Our teams enrolled patients presenting with history of acute febrile illness (1-5 day duration for most participants); safely took samples to test for corona, paramyxo, flavi, arena, filo, and influenza viruses; and administered questionnaires to collect data on risk factors for zoonotic viral transmission. Patients reported a range of symptoms in addition to fever, including headache (77%), cough (65%), abdominal pain (29%), muscle and/or joint pain (24%), or a combination of these symptoms. Symptoms did not vary by age or gender of the patients.

The majority of patients enrolled in the project were students (NNDHCs: 35%; 37MH: 25%) followed by agricultural workers (NNDHCs: 28%; 37MH: 9%) and non-animal business workers (NNDHCs: 8%; 37MH: 45%). Among the agricultural workers, the participants were most commonly engaged in crop production (96%) and to a lesser extent animal production for their primary livelihood.

CLINIC-BASED SURVEILLANCE

PARTICIPANT CHARACTERISTICS ACROSS ALL SURVEILLANCE SITES

SITES	COMMUNITY-BASED SURVEILLANCE	CLINIC-BASED SURVEILLANCE	
	Nkoranza North District (n=264)	Nkoranza North District* (n=319)	37 Military Hospital (n=47)
GENDER (FEMALE)	130 (49%)	132 (41%)	24 (51%)
GENDER (MALE)	134 (51%)	187 (59%)	24 (51%)
AGE** (YEARS)	43 (6-85)	19 (2-81)	30 (3-75)

*Nkoranza North District Health Centers 1 & 2
**median and range

COMMUNITY-BASED SURVEILLANCE

From 2017-2018, 264 participants were enrolled in our community-based surveillance activities at the Nkoranza North District site using systematic random sampling. The majority of participants (73%) were engaged in crop production and also raised domestic fowl (chickens and ducks), sheep, and pigs, primarily for household consumption. These animals are raised in extensive semi-scavenging systems where animals free-range during the day and return to mixed animal outdoor enclosures at night. Almost all of the crop farmers (98%) reported crop raiding by animals, primarily by non-human primates, rodents, bats, and wild birds. With the exception of non-human primates, deterrents were common and included trapping, shooting, and poisoning the animals to mitigate loss of crops. 62% of individuals reported an outbreak of disease in animals during the past year (poultry and sheep), yet only 18% indicated that sick animals were treated, quarantined, or culled. In addition, night soil (human excrement) and manure from poultry and sheep were commonly used as fertilizer (61%: 48/79), which if unprocessed, can also be a source of pathogens.

Nearly all respondents reported animals entering their homes (99%), including non-human primates (92%), domestic fowl (88%), sheep (79%), rodents and shrews (29%), and bats (5%). In addition, 74% of respondents consumed food that had been handled or damaged by animals and 70% observed animal feces and extreta (from poultry: 56%; non-human primates: 36%; rodents: 21%) in or near food sources. Over half (52%) of the community members said household food was not stored in closed containers, facilitating access by rodents and non-human primates.

Hunting was not commonly reported (12%) in these communities; however, rodent and bat hunting were observed at the site and 50% of individuals indicated that bushmeat was locally available. In addition, several individuals reported eating animals that they found ill (30%) or dead (13%). Further, over half of the respondents (54%) either did not know or did not perceive any risks of disease transmission from wounds when butchering an animal, which might explain why 19% of people reported that they do not take any preventive measures or seek treatment from a doctor when injured during animal butchering.

(Above, top-bottom) PREDICT conducted community outreach to increase awareness of zoonotic diseases and discuss the goals of the project; community members.

PHOTOS: PREDICT/Ghana

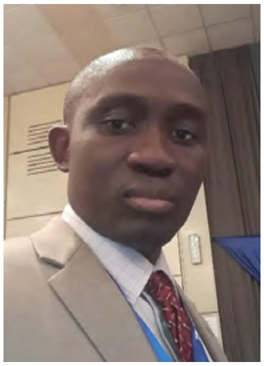
RISK COMMUNICATION

Given the zoonotic disease risk associated with bat contact that was identified through PREDICT's surveillance activities, we conducted community outreach campaigns in the Nkoranza North District communities to increase awareness and promote risk reduction strategies. Our teams, led by trusted Health Promotion Officers from the Ghana Health Service and a local Wildlife Conservation Officer, held a risk reduction campaign using materials developed by the PREDICT project, including PREDICT's behavior change communication and risk reduction resource *Living Safely with Bats*. More than 500 community members participated in this event to learn about bats, their important contributions to ecosystem services, and strategies for reducing risk of exposure to bat-borne zoonoses, while ensuring conservation of the bats. The outreach focused most intensively on how to exclude bats from entering homes in the villages, given this type of bat contact was brought to light through PREDICT's work in the community.

STRENGTHENING CAPACITY

PREDICT provided a range of training opportunities designed to strengthen existing capacities in disease surveillance and risk assessment for current and emerging zoonotic viral threats. PREDICT team members from government and university partner organizations across the human, environmental, and animal health sectors participated in local, regional, and international workshops as well as field-based and laboratory-based exercises to strengthen One Health networks and enhance knowledge and skills required for a One Health Workforce.

For example, joint on-the-job trainings were provided that reinforced concepts and skills in biosafety, safe capture and sampling of wildlife, specimen handling and maintenance of a cold chain, virus detection using consensus PCR, and disease outbreak response. In addition, trainings developed to strengthen risk assessment capacities were supported, including phylogenetic analyses and spatial risk assessment and modeling. PREDICT team members who have participated in these trainings are now sharing knowledge and skills with colleagues in the government and at the university through a "train-the-trainer" approach.



"PREDICT has significantly expanded the scope of disease surveillance in Ghana to include a focus on zoonotic diseases and an improved ability to detect novel zoonotic viruses that may be the cause of undiagnosed illnesses in Ghana."

–Dr. Franklin Asiedu-Bekoe,
Head of Disease Surveillance,
Ghana Health Service

OUTBREAK PREPAREDNESS & RESPONSE

PREDICT responded to a request by the Ghana government to support the investigation of a suspected Lassa Fever case in Ghana in March, 2018. Based on consultation with Ghana Health Service regarding locations where the deceased patient was residing during the 30-day period prior to illness, the team conducted field investigations at two sites: Adenta and Ashaiman New Town.

In collaboration with the Ghana Health Service and with support from FAO, PREDICT personnel from the Wildlife Division of the Forestry Commission and Veterinary Services Directorate, conducted the field investigations, safely sampling rodents in Adenta and Ashaiman New Town. In total, 52 rodents were sampled and tested for Lassa fever. The team also administered questionnaires in the local community. The government of Ghana views this effort as a One Health success story where personnel representing the three ministries worked collaboratively to investigate the circumstances of the case, assess rodent reservoirs of the virus around the communities, and evaluate human practices that could put this community at greater risk of exposure. PREDICT also worked with the Ghana Health Service and the School of Public Health to educate the community on Lassa fever and strategies to reduce their risk.

PRACTICAL IMPLICATIONS

PREDICT's work strengthened One Health platforms in Ghana through enhancing capacities in One Health surveillance, detection of emerging viral zoonoses, and multi-sectoral collaboration during disease outbreak investigations. The project established mechanisms to sustain One Health approaches through buy-in from stakeholders on the value of multi-sectoral partnerships in disease surveillance and disease outbreak investigation, protocols for detection of emerging viruses, and sharing of animal and human disease surveillance data. This is evidenced by the uptake of PREDICT's virus detection protocols in laboratories used by the government to investigate undiagnosed causes of illness in humans and animals and the government's designation of sentinel sites for zoonotic disease surveillance in the country.

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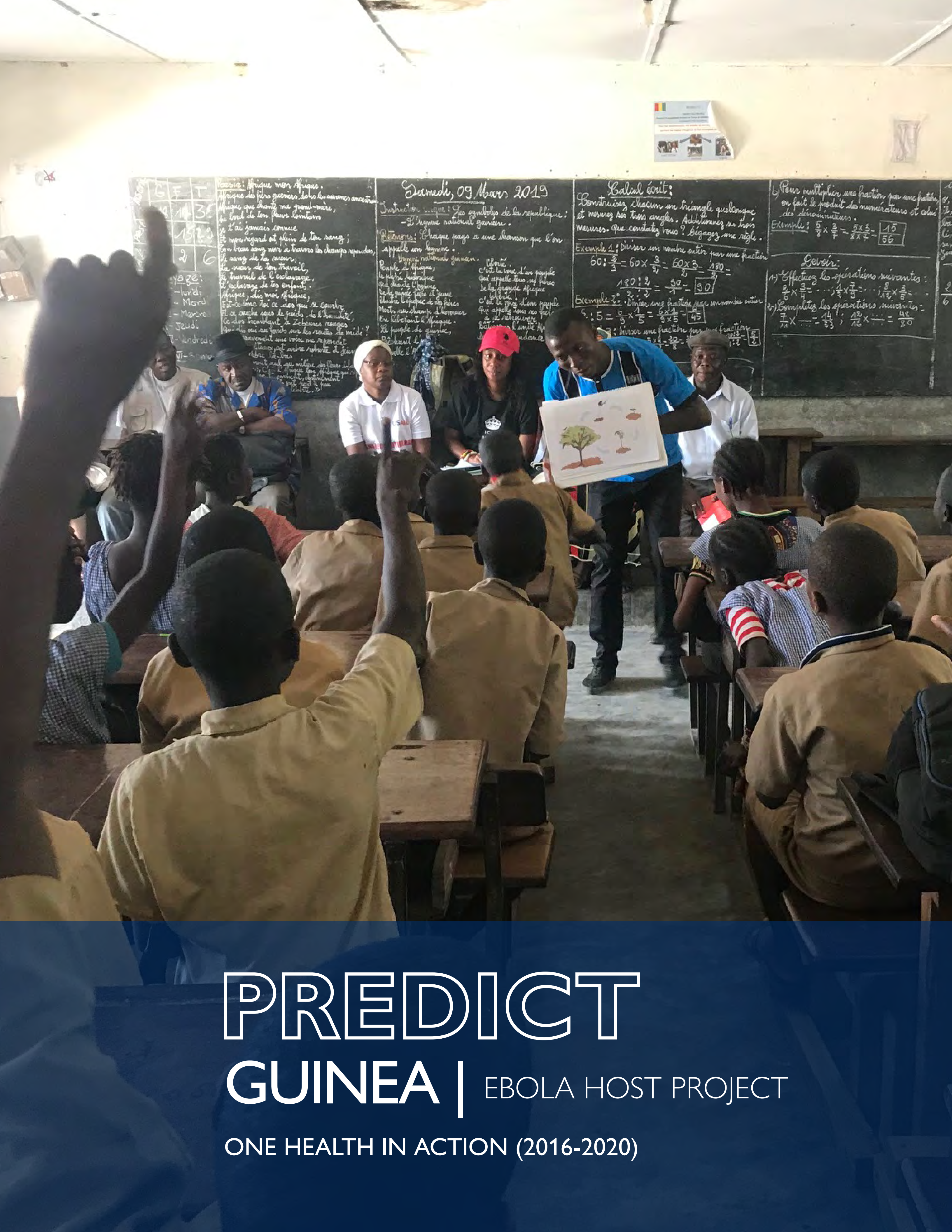
SPECIAL FEATURE

PEAK SEASON FOR VIRAL SHEDDING AT URBAN FRUIT BAT ROOSTS

Our team collaborated with scientists from UC Davis and Tanzania to assess the seasonality of coronavirus (CoV) shedding by the straw-colored fruit bat (*Eidolon helvum*) in large urban fruit bat roosts. We non-invasively collected fecal samples from the bats monthly over an annual cycle at two bat colonies: one at the 37 Military Hospital in Accra, Ghana and one in Morogoro, Tanzania. In parallel, we collected data on the roost sizes and precipitation levels and established the reproductive periods through the year (e.g., birth pulse, weaning of pups, etc.). The results suggest an association between the reproductive cycle of the bats and CoV shedding, with an approximately four times higher proportion of positive fecal samples during the pup weaning period compared to other periods of the year.

Read more about this study at **bit.ly/2O2creo**



PREDICT

GUINEA | EBOLA HOST PROJECT

ONE HEALTH IN ACTION (2016-2020)

Lessons learned from Ebola

Guinea, a country of approximately 12.9 million people on the Atlantic coast of West Africa, is characterized by several different climatic zones, largely due to great geographic diversity. There are more than 1,300 rivers in the country. Many major rivers, such as Niger, Senegal (Bafing), The Gambia, as well as their main tributaries have their sources in Guinea, making this country the "Water Tower" of West Africa. Guinea has the largest reserves of bauxite in the world and the largest untapped reserves of iron ore, gold and diamonds. Despite its significant natural resource potential, the Guinean economy remains very fragile and dependent on bauxite and agriculture.

The majority of Guineans work in the agricultural sector which employs more than 75% of the country's active population (24% of GDP). More than half (55.2%) of Guineans live below the poverty line. The rural environment in Guinea continues to be preserved thanks to a low population density and limited industrialization. This intersection of biodiversity, poverty, and rural livelihoods dependent on agriculture, subsistence hunting, and extractive industries presents multiple opportunities for viral spillover and spread across the animal-human interface, as well as significant challenges for disease prevention and control.

In 2013, somewhere in the Forest Region of Guinea, one of these spillover events presumably sparked by human contact with an infected bat, spread across the Guinea-Sierra Leone-Liberia border region and grew into the largest Ebola virus epidemic in history, leaving over 28,000 people infected and more than 11,000 dead, with 2,544 deaths in Guinea alone. This outbreak had devastating impacts on Guinea's economy and health infrastructure and dramatically affected the livelihoods of Guineans.

The West Africa Ebola epidemic catalyzed investments to identify the animal source, or reservoir, of Ebola to prevent future outbreaks. It has been >40 years since the Ebola virus was first discovered in Central Africa and the scientific and health communities continue to search for the source. However, no standardized, large-scale, longitudinal and multi-country study targeting multiple potential reservoir hosts of Ebola virus had ever been conducted. Rising to the occasion, the USAID PREDICT project designed and implemented the Ebola Host Project in Guinea, along with neighboring Sierra Leone and Liberia, to find the animal source of Ebola and other devastating filoviruses, and to investigate human behaviors associated with viral spillover.

In Guinea, the PREDICT project's primary goal was to identify the wildlife hosts of Ebola virus, investigate the distribution of the virus, and assess and characterize risks for future spillover and emergence. In partnership with the Government of Guinea, PREDICT also strengthened health security by supporting improvements in national capacity for wildlife disease surveillance and bolstering disease detection capabilities. Through analysis of project data and findings, the PREDICT project was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats. Our project's legacy is prominent as our partners continue to use PREDICT as a model to promote One Health and engage with at-risk communities, and our team of One Health professionals continues to work on current national health security challenges, including COVID-19.

LOCAL PARTNERS

- Institut de Recherche en Biologie Appliquée
- Institut National de Santé Publique (INSP)
- Institut Supérieur des Sciences et de Médicine Vétérinaire de Dalaba
- Laboratoire de Fièvres Hémorragiques en Guinée
- Ministère de l'Elevage et de la Production Animale
- Ministère de l'Enseignement Supérieur et de la Recherche
- Ministère de l'Environnement, de l'Eau et des Forêts
- Ministère de la Santé et de l'Hygiène Publique

>80

DEVELOPED the One Health Workforce by training more than 80 people in Guinea.

>4.7K

OPERATIONALIZED One Health surveillance and sampled over 4.7K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Laboratoire de Fievres Hemorrhagiques
- UC Davis One Health Institute

15,170
TESTS

10
PREDICT

6
KNOWN

DETECTED 16 unique virus in both animal and human populations.

MICHEL KOROPOGUI

Biologist, *Laboratoire de Fièvres Hémorragiques en Guinée*

"I really liked the different trainings received from PREDICT; the level of the trainer was very good. They allowed me to acquire knowledge on good laboratory practices as well as on performing basic PCR analyses. I would like PREDICT to continue giving its training. They gave me confidence in myself and in the future, but also they succeeded in making me want to learn and go far in virology research!"

MOHAMED IDRISS DOUMBOUYAO

Veterinarian, *Ministère de l'Elevage et de la Production Animale*

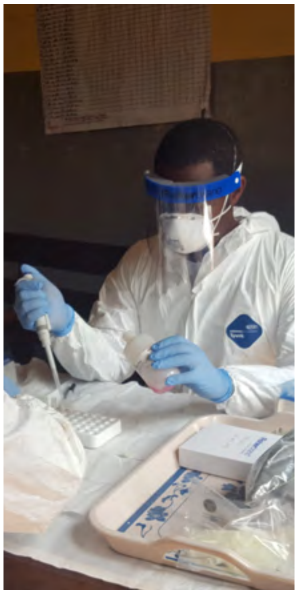
"Before, I was an office veterinarian. Thanks to PREDICT, I became a field veterinarian. This allowed me to deepen my knowledge in biosecurity, and techniques of capture and wildlife sampling. PREDICT also allowed me to have a solid knowledge of bats and rodents in general. I have always dreamed of research in the field of veterinary public health and PREDICT came to clarify my vision in this field. If I succeeded in achieving my ambitions, it would allow me to help my country, Guinea and, why not, the whole world, to cope with emerging and re-emerging zoonotic diseases in the context of One Health which I now master thanks to PREDICT."

ACHIEVEMENTS

- Safely and humanely sampled 4,754 animals and collected >28,000 samples
- Interviewed >340 individuals considered at-risk for viral spillover
- As proof-of-concept for PREDICT's approach, detected an entirely new species of ebolavirus (*Bombali ebolavirus*) in insect-eating bats; this virus was first detected by the PREDICT team in Sierra Leone and then later in Guinea; this is the first time an ebolavirus was discovered before causing human or animal illness or death
- Following the detection of Bombali virus, conducted a large-scale risk reduction and behavioral change communication campaign focused on the human-bat interface and reached >2,900 adults and >3,800 school children and high school students across 55 at-risk communities
- Supported the Global Health Security Agenda (GHSA) and contributed to improving national capacities for prevention, detection and response
- Informed national prevention and control strategies through evidence-based insights into the dynamics of viral spillover and spread and practical recommendations for risk reduction
- Supported COVID-19 response efforts by providing technical assistance to risk communication strategies and for community engagement and outreach

ONE HEALTH SURVEILLANCE

Finding the elusive wildlife hosts of ebolaviruses and other potential viral threats



In collaboration with in-country partners, an extensive surveillance program was implemented in Guinea to successfully identify the animal source and reservoir of Ebola virus and other closely related filoviruses (ebolaviruses and marburgvirus). At 17 sites across the country, mainly in the forest region (Kissidougou, Guéckédou, Macenta and N'Zérékoré), biological specimens were collected from >4,700 animals (bats, rodents, nonhuman primates, livestock, and domesticated animals such as dogs, cats, goats and sheep).

VIRUS DETECTION

In partnership between the Laboratoire de Fièvres Hémorragiques en Guinée (Viral Hemorrhagic Fever Laboratory of the University Gamal Abdel Nasser of Conakry) and the One Health Institute Laboratory at the University of California, Davis, samples from >4,500 animals (primarily bats, but also rodents/shrews, dogs, pigs, and goats) were safely tested for the presence of Ebola or other related filoviruses, along with four other priority virus groups considered high-risk pandemic threats (coronaviruses, paramyxoviruses, flaviviruses, and influenza viruses). A total of 16 viruses were detected in 29 animals, six of which are known viruses, and 10 are new (previously unknown) viruses.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Filovirus	Bombali virus (BOMV)	Angolan Free-Tailed Bat	Houndonin, Sangardo	3	3	0
Coronavirus	PREDICT_CoV-70	Halcyon Horseshoe Bat	Wondero	2	0	2
	PREDICT_CoV-109	Unidentified Rhinolophus Bat	Wondero	1	0	1
	PREDICT_CoV-115	Noack's Roundleaf Bat	Wondero	1	0	1
	PREDICT_CoV-116	Abo Butterfly Bat, Unidentified Miniopterus Bat	Wondero	3	0	3
	Bat coronavirus Hipposideros	Noack's Roundleaf Bat, Variegated Butterfly Bat	Wondero, Missira	4	0	4
	Chaerephon bat coronavirus/Kenya/KY22/2006	Gambian Epauletted Fruit Bat, Peter's Dwarf Epauletted Fruit Bat, Variegated Butterfly Bat	Missira	8	0	8
	Coronavirus 229E (Bat strain)	Noack's Roundleaf Bat	Wondero	1	0	1
	Eidolon bat coronavirus	Angolan Fruit Bat, Egyptian Fruit Bat	Wondero	2	0	2
	Kenya bat coronavirus/BtKY56/BtKY55	Angolan Fruit Bat, Noack's Roundleaf Bat, Unidentified Miniopterus Bat	Wondero	3	0	3
	Kenya bat coronavirus/BtKY66/65/63/60	Abo Butterfly Bat, Unidentified Miniopterus Bat	Wondero	2	0	2
Paramyxovirus	PREDICT_PMV-15	Peter's Dwarf Epauletted Fruit Bat	Missira	1	0	1
	PREDICT_PMV-181	Noack's Roundleaf Bat	Wondero	1	0	1
	PREDICT_PMV-182	Unidentified Miniopterus Bat	Wondero	1	0	1
	PREDICT_PMV-184	Unidentified Miniopterus Bat	Wondero	1	0	1
	PREDICT_PMV-185	Variegated Butterfly Bat	Missira	1	0	1
Total				**29***	**3**	**26***

*Numbers do not total due to individuals being co-infected with multiple viruses

NEW EBOLA VIRUS IN MOLOSSID BATS

Two bats from the free-tailed bat family (Molossidae; *Mops condylurus*) tested positive for *Bombali ebolavirus* by consensus PCR and were confirmed by sequencing, with an additional individual testing positive for *Bombali ebolavirus* by real-time PCR. Analysis of the genetic sequence information confirmed that this new virus is not the Ebola virus (*Zaire ebolavirus*) which caused the 2014 epidemic in the West Africa region. However, as an Ebola virus, Bombali virus should be considered a potential threat to human health (and possibly animals) until proven otherwise.

Bombali ebolavirus was first detected in molossid bats by the PREDICT team in Sierra Leone and since that initial detection this new virus has been identified in Guinea and as far east as southern Kenya. In Guinea, the bats in which Bombali was detected were sampled in May and June 2018 in the prefectures of Guéckédou and Kissidougou, Administrative Region of N'Zérékoré, and were captured near human dwellings. These bats are small insectivores (body weight <30g) and prefer to live in human dwellings or in other buildings and trees. Their proximity to people and evidence that this bat family is the currently known host of this new ebolavirus represents a real risk for viral spillover.

ADDITIONAL FINDINGS IN BATS

We detected 10 different coronaviruses in 25 bats and five paramyxoviruses in five bats. Two bats exhibited co-infection with two coronaviruses and four bats exhibited co-infection with a coronavirus and paramyxovirus.

Of the known and new viruses we detected, there were no viruses with the exception of Bombali virus described above that pose an immediate public health concern. However, we did detect four betacoronaviruses in bats (three known and one new betacoronavirus), which given the recent emergence of betacoronavirus SARS-CoV-2 causing the COVID-19 pandemic, additional investigation into the ecology, evolution, and global distribution of betacoronaviruses in wildlife, especially bats, is warranted.

We also detected an alphacoronavirus, Coronavirus 229E (Bat strain), in one Noack's Roundleaf Bat. While this particular strain infects bats, there is a strain that infects humans, Human Coronavirus 229E, which is known to cause respiratory illness in people. However, there is no indication that the detection of this virus strain in bats poses any public health concern.

SPECIAL FEATURE

INSIGHTS ON THE GEOGRAPHIC DISTRIBUTION OF MOLOSSID BATS IN WEST AFRICA

In direct response to the detection of Bombali virus in molossid bats and in recognition of the limited data available regarding bat distribution in the region, the Government of Guinea requested assistance identifying areas in the country and greater West Africa region at highest risk for viral spillover from bats. In response, we developed a spatial distribution model to identify areas that are ecologically suitable for habitation of Molossidae bats, such as *Mops condylurus* (Angolan free-tailed bat) and *Chaerephon pumilus* (Little free-tailed bat), that were found to harbor Bombali virus in Guinea and Sierra Leone. Using PREDICT data, our model identified areas in the region that are suitable for habitation and where the bats may be present at higher densities, resulting in increased human contact and possibly higher viral spillover risk. Tools such as this model can assist the Governments of Guinea, Sierra Leone and Liberia to better target wildlife surveillance and community-based risk reduction activities.

Learn more **bit.ly/3fH56Nw**.

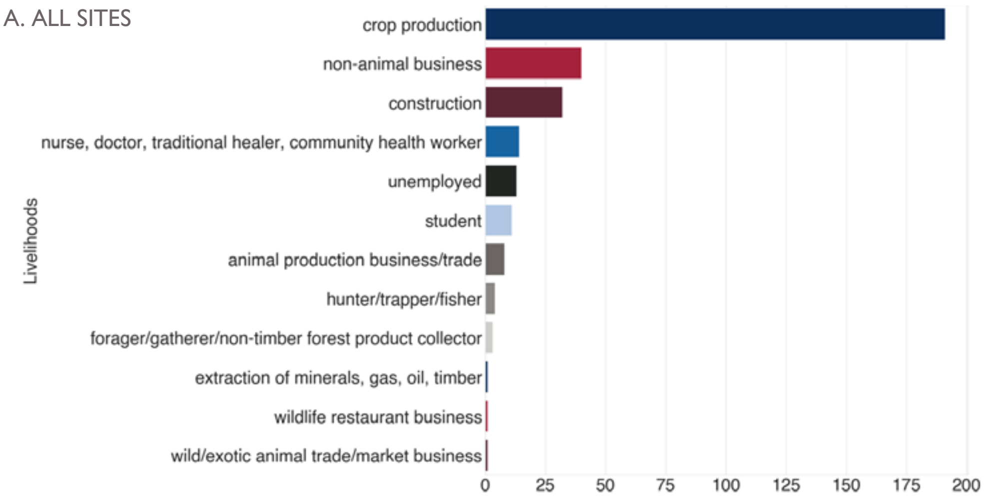
IDENTIFYING BEHAVIORAL RISKS FOR VIRAL SPILLOVER & SPREAD

Between August 2018 and January 2019, the PREDICT team interviewed 335 people using a structured questionnaire across six sites (three rural, 3 urban) in four prefectures impacted by the 2013-2016 West Africa Ebola epidemic: Macenta, Guéckédou, Kissidougou, and N'Zérékoré. For comparative purposes, questionnaires were also completed by 48 people living in a rural community in Macenta that did not have any reported cases of Ebola during the outbreak.

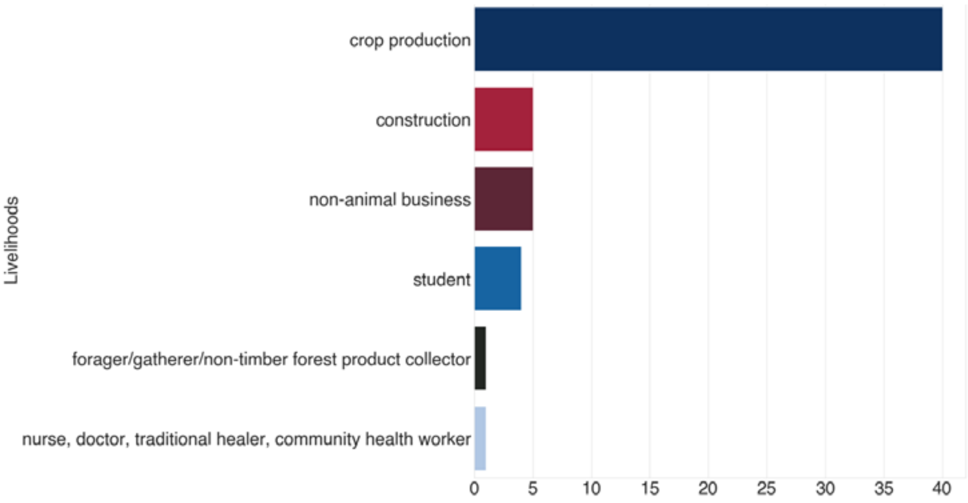
LIVELIHOODS & ANIMAL-HUMAN CONTACT

Participants described their livelihoods, health, and interactions with animals before, during, and after the outbreak. Of those enrolled in our study, 54% of respondents were male and 46% were female, and the mean age of participants was 39 years. Eighty-eight percent of participants had lived in their community for more than 10 years, most of which worked within crop production (Figure 1a, b, and c).

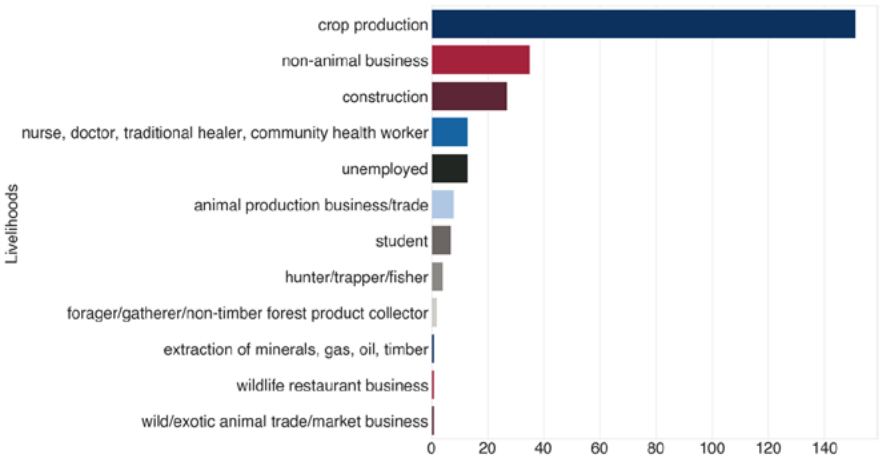
1A. ALL SITES



1B. NON-EBOLA AFFECTED COMMUNITIES (MACENTA PREFECTURE)



1C. EBOLA AFFECTED COMMUNITIES (ALL ENGAGED PREFECTURES)



FIGURES 1a, b, and c. ***Reported livelihoods of participants across all sites (1a), within the non-Ebola affected community in Macenta prefecture (1b), and within the Ebola affected communities across all engaged prefectures (1c).***

Half (56%) of people interviewed in Ebola affected regions handle or raise live animals, with poultry and swine being the most commonly kept livestock. Participants regularly reported poultry entering their home, providing an opportunity for fecal contamination of the living space, including food and water resources. Slaughtering of animals was more commonly conducted by men, and differences in animal taxa were observed among the sites, with a higher diversity reported in Djomankoidoi, Macenta prefecture. The majority of people (89%) were worried about disease in live animal markets before, during and after Ebola. Of the remaining minority of respondents, 6% reported never having been concerned, and 4% reported becoming worried about disease risk in markets during or only after the outbreak.

When comparing participant responses between Ebola affected and non-affected communities, there were no significant differences in behaviors hypothesized as risky for animal contact or for zoonotic disease transmission. This could be explained by the fact that in both Ebola affected and non-affected sites, participants were reluctant to admit engaging in high-risk activities that were observed during community engagement and animal sampling. For example, very few people mentioned that they had contact with bats although our teams observed bats in their houses while conducting the questionnaire. While these are the first findings on behavioral risk for viral spillover in the Forest Region in Guinea, it is important to conduct further ethnographic and participatory investigations that first seek to gain trust with the communities and then work to explore the full social and cultural dimensions of emerging disease risk.

HUNTING & DISEASE RISK

Because hunting of wild animals is a high-risk disease transmission interface due to the intimacy of contact, potential injury, and exposure to bodily fluid during capture, handling, and slaughtering, the project targeted individuals who reported hunting for interviews using the questionnaire. A total of 24 people across four sites (Djomankoidoi, Guéckédou, Kissigougou, and N'Zérékoré) indicating hunting as an activity and were asked additional questions about their hunting behavior. Rodents, birds, and non-human primates (NHP) were the most commonly hunted wildlife taxa (Table 3), with 92% of people hunting more than one group. Animals were predominantly hunted or trapped for consumption at home. Of the nine NHP hunters, all reported selling the animals for food and also eating them at their own homes. In the year prior to being interviewed, all hunters reported being exposed to animal blood, being scratched/bitten, and reported observing an outbreak of disease in wildlife. Despite this and as expected in these low resource communities, personal protective equipment was infrequently used and mostly consisted of footwear and/or clothes which provide inadequate and insufficient protection against disease transmission.

WILDLIFE TAXA	PERCENTAGE OF HUNTERS (n = 24)
RODENTS/SHREWS	92
BIRDS	72
NON-HUMAN PRIMATES	38
BATS	33
PANGOLINS	25
UNGULATES	13

TABLE 3. ***The most commonly hunted wildlife taxa reported by the high-risk subset of individuals interviewed***

EBOLA IN THE COMMUNITIES

Despite conducting interviews several years after the 2013-2016 Ebola outbreak, people in the communities where individuals were infected and died from Ebola virus were largely unaware of the cause (74%) (Figure 2). Only 10% of participants knew that contact with sick or deceased people could transmit Ebola virus, and 5% thought contact with animals (wild and domestic) caused Ebola sickness. These findings suggest that community outreach and education at the time of the outbreak was ineffective, with an obvious need for education on the risks associated with intimate contact with sick people and animals. Additional behavioral interventions led by community health workers, including integration of the PREDICT project's risk reduction and behavior change communication resource *Living Safely with Bats* with education programs and other disease prevention and control strategies are worth further investment.

Of those interviewed, only four were diagnosed with Ebola virus during the outbreak. All four individuals had contact with sick people and/or their bodily fluids and believed this was the source of their illness. They all reported going to the Ebola center because it was the "right thing to do". As the outbreak was widespread in these communities, we did not identify any specific behaviors associated with risk of Ebola infection. However, we did identify risky behaviors of general zoonotic disease transmission, especially from food-borne illness as all respondents reported eating undercooked meat, predominately swine, but also poultry, goats and sheep. Hunting and bushmeat consumption is also a concern. Cooking meat provides an element of protection against disease, killing many pathogens of harm to humans and should be incorporated into outreach and awareness programs.

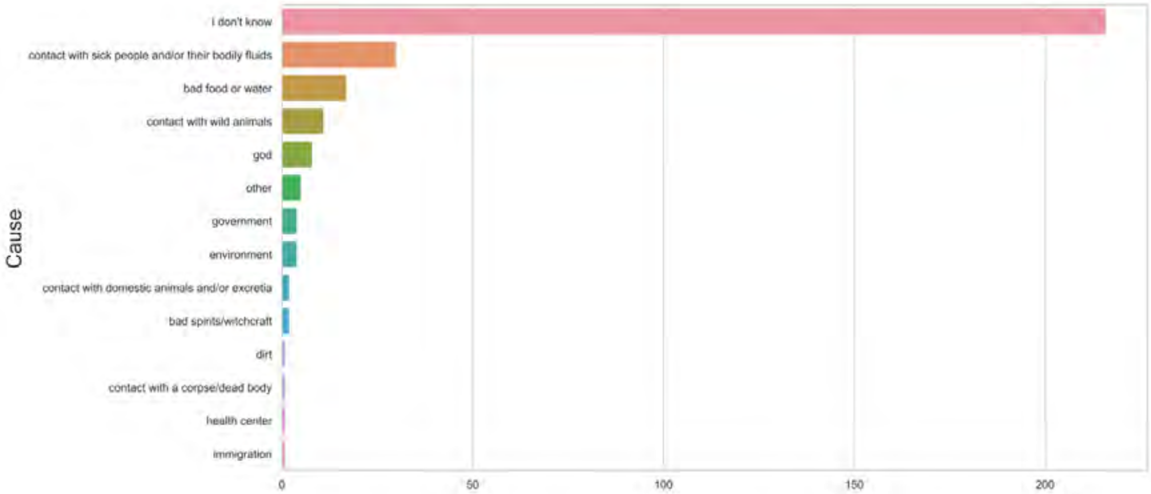


FIGURE 2. ***Participant opinions on the cause of the 2013-2016 Ebola outbreak in their community***

RECOMMENDATIONS & NEXT STEPS

Our findings, though preliminary, are important for shedding light on high-risk communities for viral spillover and spread in Guinea's Forest Region. Going forward, we recommend investments in the following three areas:

1. Community outreach, education, and behavior change communication campaigns on Ebola and other priority zoonotic diseases
2. Targeted educational interventions that balance health and conservation goals while incorporating messaging on risks and prevention of disease transmission from animals to people in the forest, fields, and around the home
3. Training on biosafety, PPE use, and safe slaughter practices for hunters, along with investments in alternative livelihood strategies to reduce disease transmission risk in this high-risk occupation.

COMMUNITY OUTREACH & RISK COMMUNICATION

In Guinea, the PREDICT team led broad and targeted (community-specific) outreach and risk communication campaigns to raise public awareness of locally relevant zoonotic disease threats such as Bombali virus in bats. Our team, consisting of the same individuals that led Ebola Host Project activities in these communities, held multiple community meetings, shared resources and flyers, conducted local radio programs, and visited classrooms in local primary schools. We also targeted high-risk groups for viral spillover such as hunters, farmers, and ranchers.

Following the discovery of Bombali virus in neighboring Sierra Leone and later in Guinea itself, our team worked with PREDICT's global network to develop and implement the risk reduction and behavior change communication resource *Living Safely with Bats*. This resource was designed as a moderated picture book, delivered by our team as trusted community partners. From October 2018 until the completion of the project in September 2019, the PREDICT team in Guinea engaged >5,000 people in the at-risk communities where PREDICT surveillance and sampling activities were based.

In addition, to reach more of the general public, our team worked with rural radio stations in Guinea to broadcast an interactive podcast program entitled "Health for All". The podcast was specifically focused on the messages in *Living Safely with Bats* and was broadcast in French along with 4 other major Forest Region dialects (Kissi, Toma, Guerzé and Malinké). The program was broadcast weekly for several months through the entire Forest Region on channels accessible to >1.9 million individuals. Building on these successes, in February 2019, following reports of a confirmed death due to Lassa Fever, Government of Guinea partners again engaged our team in risk communication efforts, focusing on daily broadcasts of the "Health for All" program in the affected region. Finally, in the wake of the COVID-19 pandemic, this team was again called into action by ministry partners to help develop and promote risk communication strategies for the current disease threat.

CAPACITY STRENGTHENING

In order to support capacity building of surveillance networks within the West Africa region and promote cross-country collaboration, the PREDICT teams participating in the Ebola Host Project (Guinea, Sierra Leone, and Liberia) engaged with each other throughout the project in trainings and online platforms to share experiences and exchange best practices. Specifically, the Guinea team traveled to Sierra Leone to participate in an animal sampling and biosafety/biosecurity training and also traveled to Liberia to gain experience and training in behavioral data collection techniques. These cross-border trainings increased knowledge and practical skills of the teams as well as building communication networks within the region. Information exchange between teams continued throughout the project, and was critical during the public release and dissemination of the Bombali virus findings to ensure all stakeholders were properly informed and engaged.

Our team also worked in collaboration with governmental and non-governmental stakeholders to develop and strengthen One Health surveillance and disease detection capacities while ensuring the sustainability of project investments. The PREDICT team organized a series of practical workshops to train laboratory technicians in hospitals and health posts, as well as animal health professionals and key workforce members in the environmental sector.

We strengthened national health security through strategic investments in zoonotic disease detection, working with the national laboratory network to establish capacity for detection of known (e.g. Ebola and Marburg) and emerging viral threats such as Bombali virus. Over the course of the project, we trained 32 national laboratory staff in the skills and techniques necessary to safely detect priority zoonoses. Trainings included laboratory safety RNA extraction, cDNA synthesis, RNA quality control and conventional PCR for filoviruses. As a result, the core foundation for both the animal and human surveillance networks in Guinea, namely Viral Haemorrhagic Fever (VHF) lab, the Central Laboratory for Veterinary Diagnosis, and the laboratory of the National Institute of Public Health (INSP), are now better connected and empowered.

Finally, as part of outreach campaigns, our team engaged with and trained 20 government agents in risk communication and community engagement and provided invaluable experience in translating difficult and challenging information on a new Ebola virus to communities often sensitive to and distrustful of authorities. These individuals are now responsible for leading risk communication strategies in their prefectural public health departments across the country, a critical role now as the country responds to the current pandemic threat, COVID-19.

ONE HEALTH IN ACTION

The PREDICT Guinea team has attended Guinea's National One Health Platform monthly meetings, giving opportunities to the Country Coordinator to disseminate data and training materials to human and animal lab partners, and to GHSA partners. These meetings were an excellent opportunity for PREDICT Guinea to transfer knowledge and capacity to Guinea's National One Health platform targeting improvements in the national zoonotic disease surveillance system. At the local and national levels, the PREDICT project continued to strengthen One Health capacity and skill development of the existing health professionals in community engagement strategy; biosafety; biological sample collection, transport, and storage; information management; and laboratory skills necessary for detection of priority zoonotic viruses.

PRACTICAL IMPLICATIONS

The PREDICT project contributed to national health security in Guinea through:

- Operationalizing One Health by developing and empowering Guinea's One Health workforce through trainings in the core skills required for surveillance, disease detection, and risk communication
- Strengthening the national laboratory system and zoonotic disease detection capacity through hands-on training of lab technicians and safe and secure testing of thousands of collected samples
- Establishing the foundation for effective behaviour change through large scale outreach and education campaigns aiming to reduce risks of viral spillover in at-risk communities.

Our achievements are best illustrated by this testimony from the Prefecture Health Director of Kissidougou:

> *"Technically-speaking, Guinea's surveillance capacity was poor before the Ebola outbreak as well as before the PREDICT Guinea-Ebola Host Project. The health system was largely unprepared for a mass disease outbreak which had shown how rapidly the disease was able to spread. This was due to several factors, including inadequate availability of human resources, a lack of support in terms of crisis and disaster preparedness. Failure to communicate effectively with the communities was another item to add to this list of inadequacies. A successful improvement of the health system was made thanks to PREDICT Guinea efforts in the Forest Region. In February 2019, we had a confirmed death from Lassa Fever in Kissidougou. There were rumors about an unknown viral spillover in the region. The Ministry of Public Health (MOPH) called upon PREDICT team to assist with community engagement and risk communication to manage rumors. PREDICT, together with prefecture health agents put risk communication and community engagement skills to manage the rumors and set life in the communities back to normal life. This collaborative, experiential-focused approach to workforce training has contributed to Guinea health system readiness and response capabilities for newly emerging diseases or outbreaks of unknown origin in the future."*

PREDICT
KENYA

ONE HEALTH IN ACTION (2014-2020)

Understanding how diseases are transmitted from animals to humans, sharing information between health professionals, strengthening capacity for rapid detection and response to viral pathogens, and identifying zoonotic diseases before they become pandemic threats.

Increasing human, livestock, and wildlife populations, expanding agricultural commercialization and animal production value chains places Kenya at significant risk for viral spillover. In addition to these drivers of infectious disease emergence, the occurrence of hemorrhagic virus outbreaks in Kenya's neighboring countries of Uganda and South Sudan have warranted concern for the risk of epidemics within Kenya due to increased cross-border trade.

Faced with these challenges, Kenya has become a leader in establishing One Health priorities and encouraging multi-sectoral coordination to address both known and emerging zoonotic disease threats. The PREDICT team worked closely with key government/ministry, university and research partners to strengthen mechanisms to detect and respond to zoonotic disease threats and build capacity in both the laboratory and field for the next generation of Kenya's One Health workforce. In addition, the team engaged interdisciplinary partners to facilitate dialogue and share One Health information across animal and public health sectors.

To strengthen capabilities for national health security, the PREDICT project in Kenya conducted One Health surveillance at high-risk, animal-human interfaces. At the same time, interviews with local community members were conducted to better understand the social and behavioral factors associated with zoonotic disease transmission.

Finally, the team strived to achieve sustainable, lasting health impacts through training, education, outreach and capacity strengthening with in-country partner institutions. The team trained 400 individuals in One Health field and laboratory skills, provided professional development for 118 students, and educated and empowered local communities to make informed decisions that benefit community health.

The project actively participated in the development of Kenya's One Health National Strategic Plan (2017-2022), effectively bridging the gap between advocacy and action related to wildlife and environmental health. Collectively, PREDICT Kenya's efforts have helped build a framework that can be used to develop effective intervention, disease prevention and control strategies at local, regional, and national scales in Kenya.

LOCAL PARTNERS

- Central Veterinary Investigatory Laboratory, Directorate of Veterinary Services-Ministry of Agriculture-Livestock
- Directorate of Veterinary Services-Ministry of Agriculture-Livestock
- Food and Agricultural Organization-Kenya
- Institute of Primate Research
- International Livestock Research Institute
- Kenya Agricultural Livestock Research Organization
- Kenya Medical Research Institute-Ministry of Health
- Kenya Wildlife Service-Ministry of Environmental Conservation
- Mpala Research Center, Laikipia County
- One Health Central East Africa
- University of Nairobi (Schools of Public Health and Veterinary Medicine)
- Zoonotic Disease Unit

>400

DEVELOPED the One Health Workforce by training more than 400 people in Kenya.

>1.8K

OPERATIONALIZED One Health surveillance and sampled over 1.8K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Kenya Medical Research Institute
- Institute of Primate Research

9,679
TESTS

1
PREDICT

1
KNOWN

DETECTED 2 unique viruses in animal populations.

JOSEPH KAMAU

Country Coordinator, *Institute of Primate Research*

"As a veterinarian, molecular biologist of infectious diseases and a zoonoses WHO-certified expert, I assumed that I had all that it takes to handle any pandemic, but my professional experience is only one side of the coin in handling an outbreak. PREDICT-2 provided the other side—the role that communities play. I've learned a lot from my interactions, including the knowledge that communities have in solving disease emergence or spread when well trained and guided, and what biosafety and biosecurity education can do to preventing disease outbreaks. I hope to use the lessons learned in many areas of my work and share them widely."

FATIMA HASSAN HUSSEIN

Research Scientist, *Institute of Primate Research*

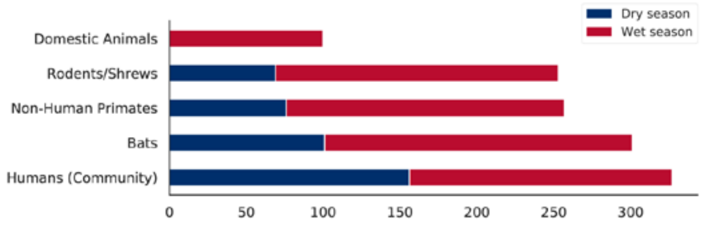
"The PREDICT project has opened my eyes from a narrow clinical diagnosis framework to a more global One Health approach by targeting emerging zoonotic viruses posing a threat not only to Kenya, but to the world."

ACHIEVEMENTS

- Trained >400 people, including the next generation of multidisciplinary One Health professionals, by developing two courses for Master's degree students in infectious disease and global health from experiences gained during project implementation
- Safely sampled >1,800 individuals (1,534 animals and 327 people) in at-risk areas for zoonotic viral spillover and spread
- Interviewed >300 people to better understand the social and behavioral factors associated with zoonotic disease transmission risk
- Detected two viruses (one known and one new) in Angolan free-tailed bats
- Contributed in drafting the One Health National Strategic Plan (2017-2022) under the stewardship of the Zoonotic Disease Unit
- Adapted the Smithsonian's National Museum of Natural History's mobile "Outbreak!" DIY exhibition materials to engage the local community, while providing context during dissemination of project results and intervention strategies
- The community health workers, nurses, and clinical officers used the PREDICT project training protocols on surveillance as part of their continued professional development – knowledge that can be used by the health workers for future surveillance and disease outbreak response
- Through partnerships with One Health Central and East Africa (OHCEA) and the University of Nairobi, strengthened disease detection, surveillance, and response capabilities of the future One Health workforce through student trainings in hands-on field and laboratory skills
- As the first zoonotic disease surveillance project in Kenya to heavily focus on wildlife virus surveillance, PREDICT Kenya highlighted gaps in communication and information-sharing between human, domestic animal, and wildlife health sectors and helped identify gaps in multi-sectoral communication and made recommendations for improved zoonotic disease prevention, detection, and response

ONE HEALTH SURVEILLANCE

In Kenya, the project's One Health surveillance was designed to train, equip, and enable the workforce to collect data and build the evidence base to understand how diseases are transmitted from animals to humans. The PREDICT team worked closely with key government/ministry, university and research partners to strengthen mechanisms to detect and respond to zoonotic disease threats and build capacity in both the field and laboratory for the next generation of Kenya's One Health workforce.



Numbers of individuals sampled by taxa group

ONE HEALTH SURVEILLANCE STRATEGY FOR NATIONALLY RECOGNIZED PRIORITY ZOONOTIC DISEASES

The PREDICT project conducted One Health surveillance to detect priority viruses and emerging diseases in animals and people at high-risk, animal-human interfaces. The team conducted interviews with local community members to better understand the social and behavioral factors associated with zoonotic disease transmission. By facilitating dialogue and sharing information between interdisciplinary partners, the project strengthened One Health platforms and knowledge exchange between animal and public health sectors.

To enable assessment of potential trends in viral spillover and spread across space, time and season, the PREDICT team conducted surveillance activities at key sites in Laikipia and Turkana Counties in Rift Valley Province over a five-year period. These sites were identified by stakeholders as locations of national importance due to their dynamic widllife-livestock-human interfaces and presence of the dromedary camel animal value chain in the region. Three land use strategies are implemented in this region: pastoralism, commercial ranching, and wildlife conservancies.

These land use systems are not mutually exclusive. For example, Laikipia County has experienced significant increases in domestic animal *and* wildlife populations over recent decades due to expansion of animal production industries and wildlife conservancy areas, creating conflict and competition for limited water and grazing resources especially during drought.

During three seasonal surveillance trips in Laikipia and one in Turkana, the PREDICT team collected over 9,130 specimens from non-human primates (NHP), bats, rodents, and camels at sites where there was a high level of interaction between humans and animals. Camel samples were collected at ranches in Laikipia, and from pastoralist herds in Turkana. Concurrent bat, rodent, and NHP samples were collected from animals in adjacent dwellings, cropland, and natural areas within close proximity to public spaces.

The team also performed human behavioral risk assessments of Laikipia residents, many of whom have direct contact with camels or wildlife and may be at risk for zoonotic disease exposure. Residents enrolled in the study also provided specimens for virus testing.

Animal samples were tested at the Institute of Primate Research (IPR) and human samples were tested at IPR as well as the Kenya Medical Research Institute. Both laboratories used PREDICT protocols to screen for known zoonotic viruses of national public health concern, like Ebola Virus, as well as for new and emerging viral threats.

HUMAN SURVEILLANCE

Through the PREDICT project's multidisciplinary One Health surveillance approach, our teams worked with local communities to learn about and better understand social and behavioral factors associated with risks of zoonotic disease transmission, focusing on the "how" and "why" of risk. Using data-driven methods, our team also worked to identify potential policies and intervention strategies that might be effective in preventing or mitigating zoonotic viral spillover and spread. Insights into behavior helps direct efforts to raise public awareness of personal and occupational zoonotic disease risks.

In Kenya, 327 people completed questionnaires on their behavior and livelihoods at high-risk animal-human interfaces and provided samples for zoonotic virus testing. Participants were community members from within four Laikipia County townships.

	Clinic-based surveillance	Community-based surveillance		
Sites/ Land Use	Commercial Ranching (n=56)	Pastoralist 1 (n=102)	Pastoralist 2 (n=100)	Commercial Ranching/ Wildlife Conservancy (n=69)
Gender				
Female	21 (37.5%)	61 (59.8%)	72 (72.0%)	40 (58.0%)
Male	35 (62.5%)	40 (39.2%)	28 (28.0%)	29 (42.0%)
Other	0 (0.0%)	1 (1.0%)	0 (0.0%)	0 (0.0%)
Age				
<18	7 (12.5%)	6 (5.9%)	28 (28%)	2 (2.9%)
18-49	43 (76.8%)	71 (69.6%)	58 (58%)	63 (91.3%)
50+	6 (10.7%)	25 (24.5%)	14 (14%)	4 (5.8%)

TABLE 1. ***Participant characteristics across all surveillance sites in Laikipia County***

SANITATION IN THE COMMUNITY

The type and frequency of high-risk behaviors related to food and water consumption and animal interaction practices varied by land use. However, high-risk activities were identified in all communities. Over 90% of people surveyed have high levels of contact with animals through pet ownership, handling and raising livestock, or sharing food/water sources with animals. The majority use an uncovered water source such as a well, pond or river and 66% do not treat their drinking water prior to consumption. These kind of intimate and frequent interactions with animals and their waste products increases the likelihood and opportunity for disease transmission from animals to humans.

Sanitation concerns were particularly observed in the pastoralist communities. Exposure to animal feces was facilitated by keeping livestock in household complexes and sharing water sources with both livestock and wildlife. Other sanitation issues included a lack of human waste locations in one pastoralist community (Pastoralist 1). Analysis revealed that having a designated location for human waste was associated with lower likelihood of illness reports among participants.

ACCESS TO HEALTH CARE

Health care access is a significant driver of disease vulnerability. Access is highly variable among communities, and is determined by distance from the community, availability of timely transportation, and cost. The pastoralist communities at greatest risk of behavioral exposure also have the least access to health services due to distance (up to 7 km to the nearest facility) and cost.

PERCEPTION OF DISEASE RISKS FROM ANIMALS

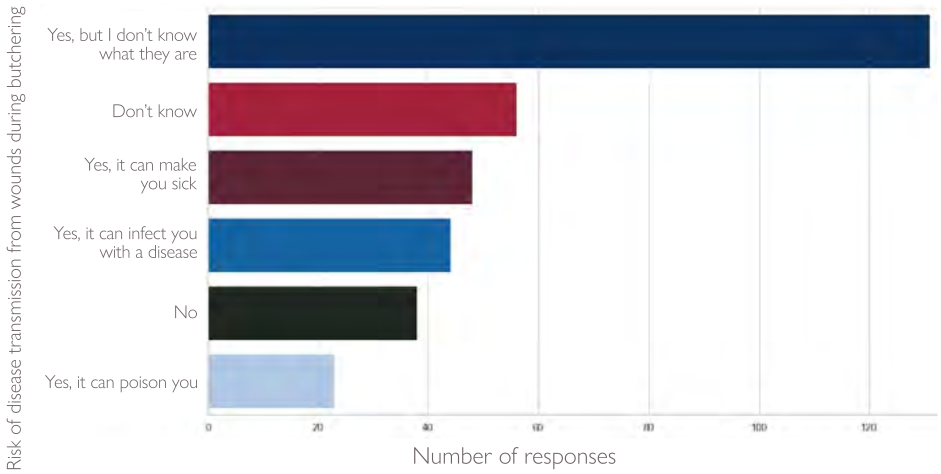
Consuming raw meat, milk, or blood from livestock are common practices in many Kenyan communities. Most people interviewed (93%) had eaten raw meat or a sick animal in the last year. These practices have been linked to outbreaks of Rift Valley Fever (RVF) and brucellosis in rural Kenya. Both diseases have devastated livestock productivity and caused human morbidity and mortalities.

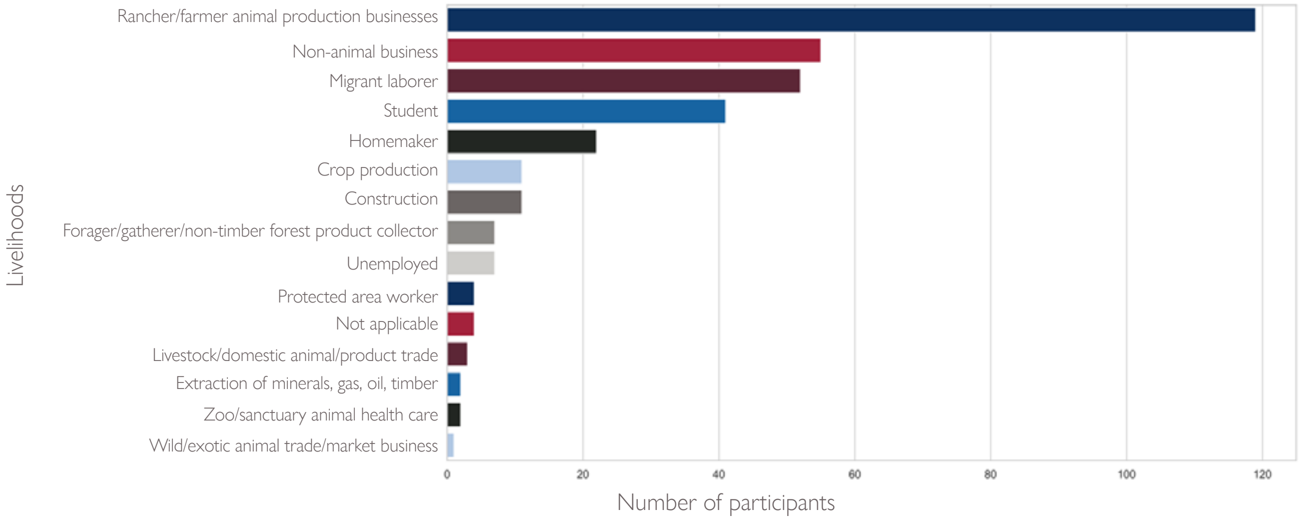
Hunting was rarely reported across all communities, but data for this behavior is unlikely to be accurate since hunting wildlife is illegal in the region. Bushmeat availability was highly reported in pastoral communities, with 76/112 people reporting that it is available to eat at or near their workplace, suggesting hunting is a common practice. A high proportion of interviewees had been scratched or bitten by an animal within the previous year but over half of the community participants (67%) either did not know or did not think there is a risk of getting diseases from open wounds when butchering animals. This explains why very few people (12%) take preventative measures (e.g. disinfecting lacerations) or seek medical treatment when injured while butchering dead animals.

OCCUPATIONAL DISEASE RISKS

Most people interviewed worked in the animal production business. Contact with infected bodily fluids or consumption of products from ill animals have previously facilitated outbreaks of Rift Valley fever in Kenya. Fifteen percent of pastoralists in Pastoralist 1 township reported an outbreak of diseases in animals during the previous year, yet only 5% said animals were quarantined or destroyed. Combined with the common practice of consuming farmed meat onsite (107/112), there is a significant risk of disease transmission from the consumption of sick animals.

Interventions emphasizing practical behaviors in industries with frequent and intimate interactions such as animal production should be explored. Community education on safe animal husbandry practices and how to identify and manage sick animals could have far reaching effects on reducing incidence of human illness. Provision and use of protective gear or clothing (e.g. gloves, face masks) to prevent wounds and contact with infected animal fluids would reduce occupational exposure, given 82% of workers in this industry are not using any personal protective equipment (PPE).





VIRUS FINDINGS IN HUMANS

The PREDICT partner laboratory, Kenya Medical Research Institute (KEMRI), safely tested oral and/or fecal samples from all 327 community participants. A total of 1,845 individual tests were conducted, and all were negative for priority virus groups (coronaviruses, filoviruses, paramyxoviruses, and influenza viruses) using consensus PCR, a broadly-reactive molecular methodology that is capable of detecting both known and unknown viruses within samples.This finding suggests that at the time of sampling, no one had an active infection, but the protocol does not assess previous exposure to viruses.

To address this issue, the team collected serum samples from 100 participants at two townships (Pastoralist 1 and Commerical Ranching/Wildlife Conservancy). Serum contains antibodies that are produced by the immune system when a person is exposed to virus in response to a previous infection. By looking for the presence of antibodies, serological methods provide an indication of past exposure to viruses. The PREDICT team used a new technology to test samples for eight viruses of concern. Among the 100 participants, people were reactive to Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, alphaviruses, and flaviviruses. These serology results are considered preliminary and samples will need further confirmation. However, these results are consistent with other studies that found high seroprevalence of flaviviruses among certain Kenyan communities. The following factors may help explain these results: the Commercial Ranching/Wildlife Conservancy community is close to rivers and a dam, providing favorable breeding habitat for mosquitoes that harbor the vector-borne viruses; wildlife have been suggested as hosts of flaviviruses, such as yellow fever and dengue viruses, and previous studies have shown that conservancy areas in Laikipia have significantly higher population densities of wildlife when compared to pastoralist areas, thus facilitating close contact and a potential transmission interface.

WILDLIFE SURVEILLANCE

Between July 2016 and May 2018, the PREDICT team safely and humanely collected samples from 911 individual animals at high-risk interfaces in Laikipia and Turkana Counties; 257 non-human primates, 252 rodents, 302 bats and 100 camels. The PREDICT laboratory at the Institute of Primate Research tested 1,651 fecal and oral swabs for priority virus groups (coronaviruses, filoviruses, paramyxoviruses, and influenza viruses) using consensus PCR, which tests for active viral infection at the time of capture and sampling.

VIRUS FINDINGS IN WILDLIFE

Two viruses were found in eight Angolan free-tailed bats (*Mops condylurus*) in Naibor. The known virus Chaerephon bat alpha-coronavirus/Kenya/KY22/2006 (Genbank Accession no. HQ728486) was detected in the oral swabs from three female bats. A novel alphacoronavirus named PREDICT_CoV-90 was detected in the oral swabs from three female bats and in fecal swabs from two male bats. There is no evidence at this time to suggest either virus poses a threat to human health. All other samples from bats, rodents, shrews, non-human primates and camels were negative for priority virus groups (coronaviruses, filoviruses, paramyxoviruses, and influenza viruses).

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	TOTAL POSITIVE INDIVIDUALS
Coronavirus	PREDICT_CoV-90	Angolan Free-Tailed Bat	Naibor	5
	Chaerephon bat coronavirus/ Kenya/KY22/2006	Angolan Free-Tailed Bat	Naibor	3
Total				**8**

EPIDEMIOLOGICAL & BEHAVIORAL RISK

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

The PREDICT project has been committed to community engagement - working with local villages and partners to raise awareness of zoonotic disease threats and strengthening capacity for prevention, surveillance, and detection. Sensitization meetings were held at all project sites over the life of the project. At these meetings, our team worked with local communities to create a dialogue and build trust for implementation of surveillance activities and a platform to communicate One Health approaches.

Implementation of outreach and engagement activities provided a forum in Kenya to empower community members to make the most informed decisions regarding their own health security on an individual level and from a community perspective. Participants included County veterinary and public health officials, One Health Workforce students, community leaders and community health and animal health workers. The PREDICT team conducted workshops to introduce the One Health approach and discuss potential sources of zoonotic diseases, behavioral risk factors, and the strategies for disease prevention, detection, and response. In addition, our team employed a variety of visual education tools to invite discussion on specific recommended mitigation strategies, while being mindful of potential conflicts between cultural or customary traditions and health safety. Further topics focused on clinical signs that should be monitored by health clinics and encouraged rapid reporting to local health officials.

Through behavioral risk characterization, we were able to identify risk factors and target zoonotic disease risk mitigation at the local community scale. In community meetings, the PREDICT team provided summaries of project findings and shared resources to raise awareness of disease prevention. Our teams communicated the importance of reducing contact with animals and their parts/products through practicing good hygiene, as well as using barrier protection against wildlife in households. Key visual education tools were shared to support specific public health recommendations, including a mobile Outbreak DIY panel exhibition developed in conjunction with Smithsonian's National Museum of Natural History, a documentary film featuring core PREDICT staff, and the behavior change picture book *Living Safely with Bats* that is designed to minimize risks of infection to bat-borne viruses such as Ebola, Marburg, and MERS coronavirus. These resources were also used to teach primary school students best hygiene practices and encourage positive and safe associations with nature and wildlife. Targeted early education of children provides a lifelong lesson with positive, cumulative effects on disease prevention for future generations.

OUTBREAK RESPONSE & PREPAREDNESS

During the avian influenza outbreak in neighboring Uganda and the Marburg alert in northwestern Kenya, the PREDICT team participated in preparedness and response discussions in partnership with the Directorate of Veterinary Services and the national Zoonotic Disease Unit (ZDU)/One Health Technical Working Group. By sharing protocols for disease surveillance, detection and response, our team provided support and expertise to help prepare for and prevent these diseases from spreading into/across the country.

ONE HEALTH WORKFORCE CAPACITY STRENGTHENING

In Kenya we strived for sustainable, lasting health impacts through training, education, outreach and capacity-strengthening with in-country partner institutions. The team trained 382 individuals in One Health field and laboratory skills, provided professional development for 165 students, and educated and empowered local communities to make informed decisions that benefit community health. The PREDICT team actively participated in the development of Kenya's One Health National Strategic Plan (2017-2022), in close collaboration with government and academic partners. This activity effectively bridges the gap between advocacy and action related to wildlife and environmental health policy. Tabletop simulations were conducted with the Zoonotic Disease Unit, in partnership with FAO and other EPT-2 partners. Participants were trained in transmission dynamics, experienced how to identify potential disease sources, and learned how to collaborate with relevant government authorities in disease response.

In collaboration with key partners, the Kenya team provided field training for zoonotic disease surveillance and public health education for community health and animal health workers. These workers are subsequently better equipped to identify and address potential sources of zoonotic disease exposure and behavioral risk factors in the community as a result of animal-human contact. Their newfound knowledge will be disseminated amongst the community members and will help identify targets for intervention and risk mitigation.

Overall, our efforts helped build a framework that can be used to develop effective intervention, disease prevention and control strategies at local, regional, and national scales in Kenya. Additionally, we increased Kenya's national laboratory capability to perform viral disease detection by partnering with and training the Institute of Primate Research (IPR) and Kenya Medical Research Institute (KEMRI). In-country expansion and self-sustainability of the workforce was achieved through cross-training when IPR hosted Central Veterinary Lab (CVL) staff for a two-week in-service training using PREDICT testing protocols to detect zoonotic diseases.

PRACTICAL IMPLICATIONS

In Kenya, the PREDICT team developed sustainable and lasting health impacts through One Health training in field and laboratory skills, capacity-strengthening in-country partner institutions, professional development of students, and education and empowerment of local communities in making informed decisions. These activities have benefited national and community health surveillance, preparedness, and response. Our activities bridged the gaps between advocacy and action and connected human, animal, and environmental health sectors. Our work can be applied towards identifying targets for intervention and developing effective disease prevention and control strategies at local, regional, and national scales.

Our key findings included a low level of awareness across at-risk communities that contact with animals can cause disease in people, and that risk mitigation strategies need to be tailored to specific sites for appropriate social and cultural fit. Targeted training of community-identified/community-based health workers provide one of the best opportunities for disseminating public health initiatives to vulnerable people living at high-risk animal-human interfaces.

RECOMMENDATIONS

Rural communities at high-risk sites throughout Kenya should be engaged in conversations that reveal underlying beliefs and behaviors influencing disease dynamics. Subsequently, public health interventions should be tailored to address the most prevalent high-risk behaviors. Educational outreach programs in one of the Kenya PREDICT sites, for example, should target food safety and animal-human interactions specifically on the **risks of eating raw meat and sick animals**.

Effective community intervention should also emphasize **training for local health workers**, who were liaisons between the PREDICT project and at-risk communities, and who can increase agency awareness by presenting information in a relevant and culturally sensitive way. Further, **implementing health safety education programs** in primary schools (the predominant level of education at one site) and at free health clinics can also be strategically targeted to reach as many residents as possible.

While high risk behaviors depended on site / land use, all communities would benefit from **enhanced water safety and waste management**. Working with communities to develop realistic and scalable solutions to improve sanitation practices would be a reasonable first step in promoting safe practices.

These outreach strategies can strengthen local response capacities and mitigate high-risk behaviors while being rooted in a One Health approach.

PUBLICATIONS & PRESENTATIONS

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- Mutinda, Mathew, et al. "Blood Biochemical Reference Intervals for Free-Ranging Olive Baboons (Papio anubis) in Kenya." *International Journal of Primatology* 40.2 (2019): 187-196. DOI: 10.1007/s10764-018-0074-2
- Yu, J., et al. "Implementation of the Smithsonian's Outbreak DIY Toolkit for local communities of Kenya and Myanmar: challenges and lessons learned." *American Society of Tropical Medicine & Hygiene.* Ft. Washington, MD. 23 November 2019. Poster Presentation.
- Zhu, Sophie, Dawn Zimmerman, and Sharon L. Deem. "A review of zoonotic pathogens of dromedary camels." *EcoHealth* (2019): 1-22. DOI: 10.1007/s10393-019-01413-7
- Zimmerman, Dawn M., et al. "Endemicity of yaws and seroprevalence of Treponema pallidum antibodies in nonhuman primates, Kenya." *Emerging Infectious Diseases* 25.11 (2019): 2147. DOI: 10.3201/eid2511.190716.

PREDICT

LIBERIA | EBOLA HOST PROJECT

ONE HEALTH IN ACTION (2016-2020)

Finding the wildlife hosts of Ebola virus

The 2014 West Africa Ebola epidemic, which infected over 28,000 people and left 11,000 dead, catalyzed investments to identify the animal source, or reservoir, of Ebola to prevent future outbreaks. It has been >40 years since the Ebola virus was first discovered in Central Africa and the scientific and health communities continue to search for the source. However, no standardized, large-scale, longitudinal and multi-country study targeting multiple potential reservoir hosts of Ebola virus had ever been conducted.

Following the epidemic, the PREDICT project initiated the Ebola Host Project in 2016 in Liberia along with neighboring Sierra Leone and Guinea. Our goals were to identify the wildlife reservoir for the Ebola virus that started the epidemic, to detect other related filoviruses, and to better understand the disease ecology of these filoviruses in the wild to develop risk reduction and prevention strategies.

Through the Ebola Host Project, the PREDICT project strengthened capacity for wildlife surveillance and disease detection in Liberia by training a local workforce in wildlife and domestic animal disease surveillance and in behavioral risk investigations to explore the social dimensions of zoonotic transmission in some of the country's most at-risk communities. The PREDICT project's animal disease surveillance team was the first of its kind in Liberia; the team put acquired One Health skills in practice and sampled >5,000 animals, primarily bats and rodents. This sampling effort resulted in the first ever detection of *Zaire ebolavirus* in a bat in West Africa, evidence that bats are an important reservoir host for this rare and deadly virus. As a result of PREDICT project investments, this highly skilled team is now well positioned to continue contributing to disease surveillance, detection, and response and provides a critical but previously absent epidemiological component to Liberia's public health surveillance system. Among those trained in core One Health skills were two Forestry Development Authority (FDA) personnel, a critical government partner institution for sustaining these capacity gains and progressing on Liberia's journey towards self reliance in the health sector.

The PREDICT project also promoted the One Health approach within the public health sector by strengthening collaborations with the Ministry of Agriculture, Food and Agriculture Organization of the United Nations (FAO), National Public Health Institute of Liberia, and the Ministry of Health. In Liberia, the PREDICT project is viewed as an important contributor to national health security making direct impacts on One Health and zoonotic disease surveillance systems. Our team supported the operationalization of the One Heath approach in-country and participated in the country's monthly Surveillance Technical Working Group meetings under the One Health Coordination Platform. These linkages provided our team the opportunity to raise wildlife disease considerations and engage with the environment, forestry, health, and agriculture authorities on potential surveillance and monitoring strategies, some leading to the formation of multisectoral investigation teams. The inclusion of wildlife monitoring in the National Animal Disease Surveillance Plan – formally identifying wildlife information as a key source for the surveillance network – and National Action Plan for Health Security represent major successes for One Health surveillance in Liberia. In addition, the PREDICT project's involvement in Liberia's One Health Coordination Platform helped to raise attention to other relevant drivers and interfaces for disease emergence such as the wildlife trade.

LOCAL PARTNERS

- ArcelorMittal (AML)
- Food and Agriculture Organization of the United Nations (FAO)
- Forestry Development Authority (FDA)
- Ministry of Agriculture
- Ministry of Health
- National Public Health Institute of Liberia (NPHIL)
- Society for Conservation of Nature of Liberia

>30

DEVELOPED the One Health Workforce by training more than 30 people in Liberia.

>5.3K

OPERATIONALIZED One Health surveillance and sampled over 5.3K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- National Public Health Institute of Liberia
- Columbia University Center for Infection & Immunity

31,120
TESTS

9
PREDICT

1
KNOWN

DETECTED 10 unique viruses in animal populations.

ACHIEVEMENTS

- Built the first-of-its-kind wildlife surveillance team in Liberia
- Detected *Zaire ebolavirus* in a cave roosting, insectivorous bat, the first discovery of Ebola in a bat in West Africa and further evidence that bats are an important reservoir host for this rare and deadly virus
- Conducted community outreach and risk communication campaigns balancing health and conservation goals that focused on sharing findings, raising awareness, and influencing behavior change
- Played a prominent role in establishing and operationalizing the National One Health Coordination Platform
- Piloted the PREDICT-developed illustrated book, *Living Safely with Bats*, in local communities

ONE HEALTH SURVEILLANCE

In collaboration with in-country partners, an extensive surveillance program was implemented in Liberia to identify the animal source and reservoir of Ebola virus and other closely related filoviruses (ebolaviruses and marburgvirus). At multiple sites across the country, biological specimens were safely and humanely collected from 5,387 animals (5,182 bats and 205 rodents).

> "There have been unanswered questions about the source of Ebola outbreaks. There was speculation that they may have originated from bats, but there was no direct evidence."
>
> –Dr. Simon Anthony
> PREDICT Pathogen Detection & Discovery Co-Lead

VIRUS DETECTION

In partnership between the National Public Health Institute of Liberia and the Columbia University Center for Infection and Immunity (CII), samples from >5,300 animals were safely tested for the presence of Ebola or other related filoviruses, along with another priority virus group, paramyxoviruses. One virus, *Zaire ebolavirus*, was detected in a Nimba long-fingered bat, *Miniopterus nimbae*. Additionally, nine novel paramyxoviruses were detected in Noack's roundleaf bats (*Hipposideros ruber*).

ZAIRE EBOLAVIRUS DETECTED AN INSECTIVOROUS BAT

One sample from a Nimba long-fingered bat (*Miniopterus nimbae*) sampled in Nimba county tested positive for *Zaire ebolavirus*. This is the first identification of Ebola virus in a bat in West Africa. To confirm the finding and sequence the genome, PREDICT's team at the CII lab utilized VirCapSeq-VERT, a new tool invented at CII that improves the sensitivity of next generation sequencing 1,000-fold. No human cases were associated with this discovery, though work continues at CII to determine if the virus detected in this bat was the same strain that caused the 2014 West African Ebola epidemic.

Long-fingered bats of the genus *Miniopterus* are small insectivorous bats (family: Miniopteridae) that are found throughout Africa. They are an agriculturally important group, as they eat insects that damage crops. These bats typically found in forests, caves, and mines and are not known to roost in people's homes or dwellings, critical information for reducing risk of contact between bats and people and for preventing Ebola viral spillover.

Following the detection of the virus, the PREDICT team worked with the Government of Liberia to proactively develop a communication and public engagement strategy. The Ministry of Health and National Public Health Institute of Liberia paired the public release of the finding with a risk communication plan balancing health and conservation goals. The outreach campaign targeted communities in Nimba county and the surrounding region and was designed to raise awareness of zoonoses and risk of disease transmission while minimizing unintended consequences and harm to bats.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	TOTAL POSITIVE INDIVIDUALS
Filovirus	Ebola virus (EBOV)	Nimba Long-Fingered Bat	Nimba county	1
Paramyxovirus	PREDICT_PMV-186	Noack's Roundleaf Bat	Nimba county	1
	PREDICT_PMV-187	Noack's Roundleaf Bat	Nimba county	1
	PREDICT_PMV-188	Noack's Roundleaf Bat	Nimba county	1
	PREDICT_PMV-189	Noack's Roundleaf Bat	Nimba county	3
	PREDICT_PMV-190	Noack's Roundleaf Bat	Nimba county	2
	PREDICT_PMV-191	Noack's Roundleaf Bat	Nimba county	4
	PREDICT_PMV-192	Noack's Roundleaf Bat	Nimba county	1
	PREDICT_PMV-193	Noack's Roundleaf Bat	Nimba county	1
	PREDICT_PMV-194	Noack's Roundleaf Bat	Nimba county	1
Total				**16**

IDENTIFYING BEHAVIORAL RISKS FOR VIRAL SPILLOVER & SPREAD

The PREDICT team examined the social dimensions driving viral spillover and spread by investigating human and animal interactions, and exploring knowledge, attitudes, and practices associated with disease transmission risk. Our team designed a study and helped develop a standardized questionnaire designed for use in all three Ebola Host Project countries. Goals of the study were to: 1) identify behavioral and ecological factors that could lead to the risk of viral spillover, amplification, and spread; and 2) determine potential targets for intervention based on this evidence. This questionnaire was developed to explore the different ways that people interacted with wild and domestic animals, the kinds of exposures individuals faced in markets, how they mitigated risk during high-contact activities such as hunting and handling bushmeat, and how they understood their own susceptibility to disease.

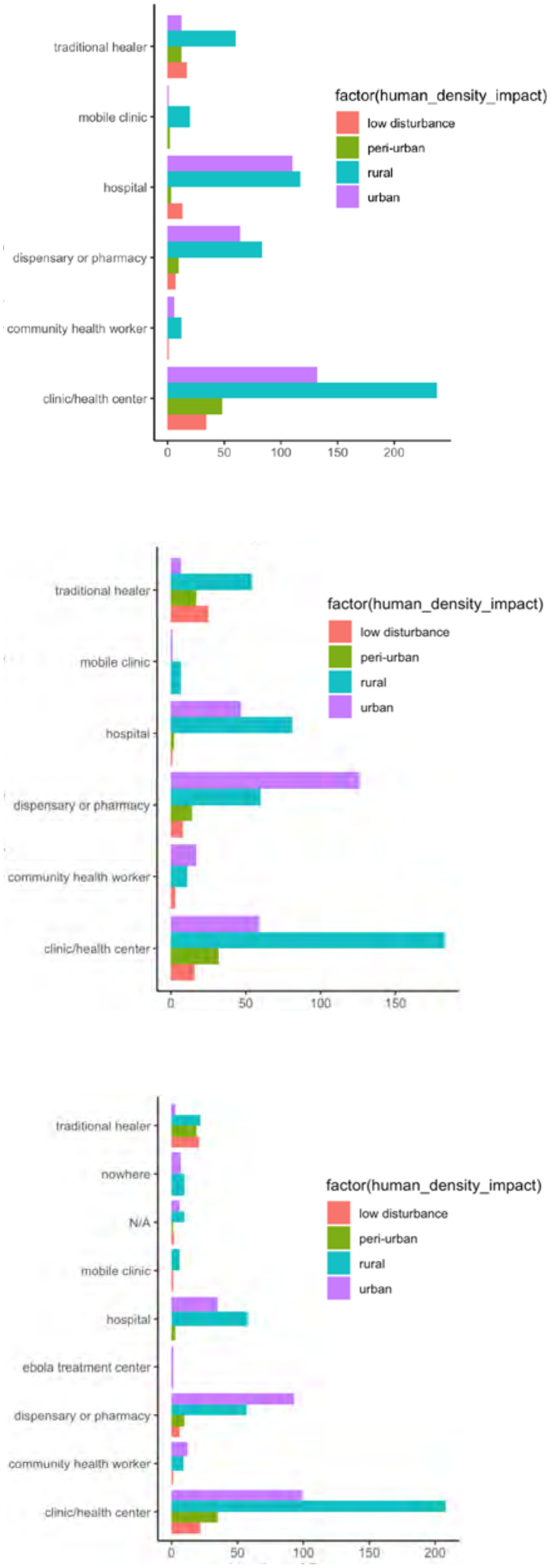
In Liberia, participants were recruited from areas affected by the 2014 Ebola epidemic and in communities where humans regularly interacted with wildlife and domestic animals (e.g. live animal/bushmeat markets), sites with a high density of species of zoonotic disease interest (e.g. bat roosts, non-human primate habitats, etc.), and control areas that had no reported cases of Ebola. These sites purposefully represented geographies ranging from urban, to rural, and pristine. We enrolled and interviewed 635 individuals, including four children, across eight counties (Lofa, Nimba, Bong, Gbarpolu, Grand Cape Mount, Grand Geddeh, Sinoe, and Montserrado).

PRELIMINARY FINDINGS

Though analysis is ongoing, our findings highlight the proximity with which many communities across the pristine-rural-urban landscape exist and interact with animals. Poultry, rodents, goats, and sheep were reported as regularly entering human dwellings, and respondents shared reports of cooking or handling recently killed rodents and ungulates. Many respondents also reported eating raw, undercooked, or smoked meats, organs, and blood – frequently rodents and ungulates. The presence of rodents in proximity to dwellings and consumption of rodents in particular presents a risk for transmission of Lassa virus, which is endemic in the region and is a recognized priority zoonotic disease.

A recurring theme that emerged in our analysis was the intractability of behavior and preference, even when tested by crisis. Respondents were asked about health-seeking behaviors for medical issues both before and during the Ebola outbreak. In general, individuals reported accessing 'formal' health services through hospitals, clinics, and health centers, though traditional care was also an important component of treatment for respondents from all geographic areas (Figures 4, 5, and 6).

When asked about the etiology of Ebola, the majority of respondents acknowledged that they did not know what caused the Ebola outbreak in their country. However, many respondents did attribute the origins of Ebola to wild animals, sick people, their bodily fluids, and domestic animal excreta (Figure 7). With regard to concerns over disease outbreaks in local live animal markets, respondents across all geographies noted a stark rise in their concern during the Ebola outbreak. In the period following the crisis, however, reported concern diminished (Figure 8).



FIGURES 4-6. ***Where individuals reported seeking health care before (top), during (middle), and after the outbreak (bottom). Respondents were able to select all applicable options.***

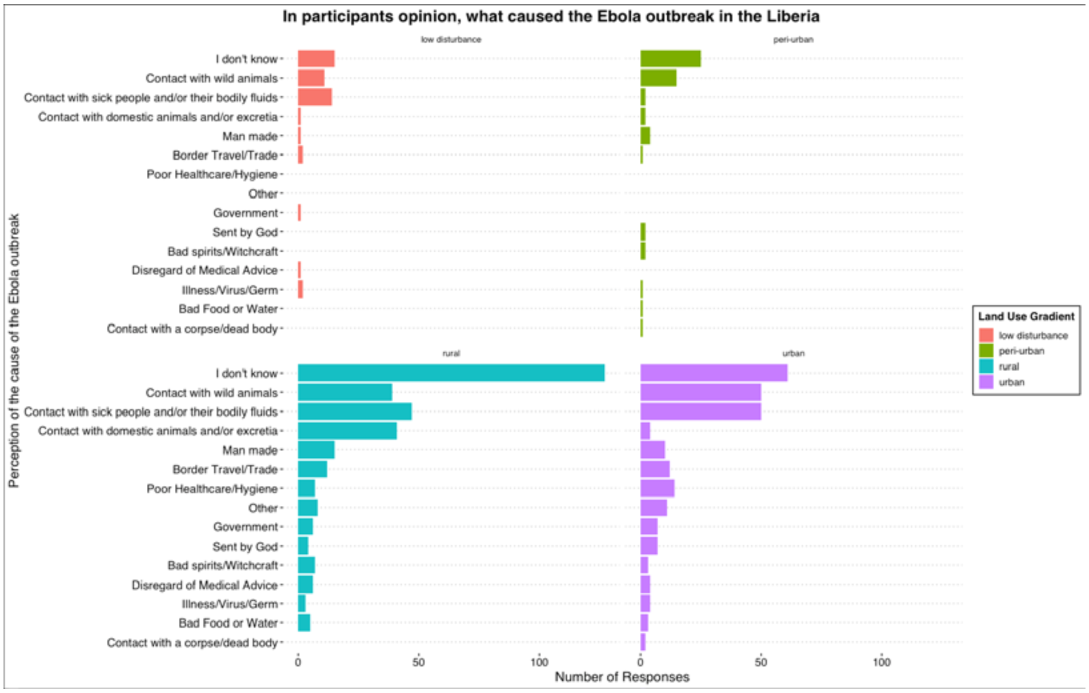


FIGURE 7. *Interviewer responses on perceived cause of the 2014 West African Ebola epidemic.*

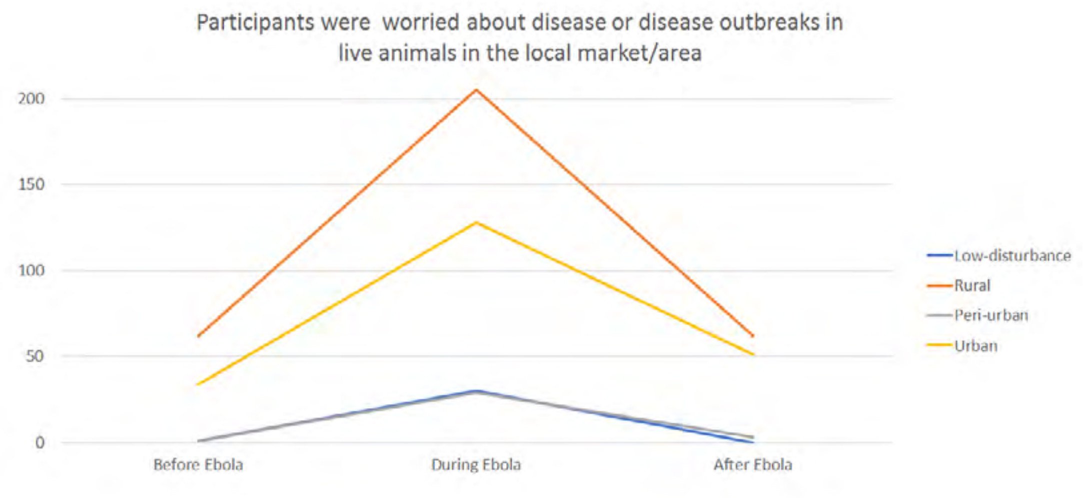


FIGURE 8. *Reported concern about disease or disease outbreaks in local live animal markets before, during, and after the Ebola outbreak.*

COMMUNITY OUTREACH & RISK COMMUNICATION

The PREDICT project played an instrumental role in establishing the One Health Coordination Platform in Liberia, making it operational by providing technical, logistical, and moral support to the effort. In addition, our staff were involved in the development of the National Action Plan for Health Security, National Surveillance and Response plans for Lassa fever and influenza, and provided technical support on many zoonotic disease related topics. Further, the PREDICT team were members of the One Health Technical Working Groups since the formal establishment of the One Health Platform providing a unique perspective, especially on conservation and emerging disease risks from wildlife.

Community engagement was critical to the success of our One Health surveillance strategy. The team in Liberia performed outreach in sometimes difficult environments, explaining the importance of the project and our approach. Our team met with community leaders to answer questions and explain the project prior to engaging in fieldwork. In communities affected by the 2014 Ebola epidemic, this was no small task. Perhaps more importantly, our team revisited all of the communities where we conducted surveillance and fieldwork to share our findings and presented the PREDICT project-developed risk reduction and behavior change communications resource *Living Safely with Bats*.

Communication was perhaps never more important than following the discovery of *Zaire ebolavirus* in a bat in northern Liberia. Upon informing government partners of the discovery, the Ministry of Health decided to announce the finding to the public. There was still pervasive societal sensitivity to Ebola which had devastated the country only five years earlier. However, the Government of Liberia believed that it was important to inform the public of their efforts to identify where Ebola came from and to provide news of the discovery along with guidance on how to reduce the risk of exposure by avoiding mines and caves where the type of bat that tested positive lives. PREDICT worked closely with government partners to develop evidence-based messaging that would help reduce risk of exposure to Ebola virus without creating fear of bats.

> “One of PREDICT’s most important achievements in Liberia was the development of the country’s first surveillance team capable of safely sampling bats and other wildlife for zoonotic viruses, including Ebola. To prevent the next outbreak, whether it’s Ebola or Disease X, it’s necessary to understand where these viruses come from. Liberia now has the ability to do that.”
>
> –Dr. Jon Epstein
> PREDICT Global Team & Ebola Host Project, Liberia Technical Lead

STRENGTHENING CAPACITY

Despite extremely limited veterinary capacity in Liberia, our team leveraged PREDICT project investments in wildlife surveillance and broader One Health skills and competencies to build a strong foundation for providing a range of field, policy, and training opportunities. For example, PREDICT project partners in Côte d’Ivoire expressed an interest in strengthening wildlife surveillance capacity, and in response, our Liberia team co-led a training with the PREDICT Côte d’Ivoire team. Held at the Abidjan Zoo in June 2019, trainers from the Liberia team provided hands-on training in bat and rodent sampling to 50 participants from a range of government partners in Côte d’Ivoire. The involvement of the Liberian team also reinforced the value of cross-border collaboration on surveillance and information sharing, contributing to wider regional preparedness. Further leveraging their training on safe and humane animal handling and One Health, our team has been called on by the Liberian government to implement and provide expertise during canine rabies vaccination campaigns.

In addition, the PREDICT team in Liberia was trained on One Health policy and evaluation, using their skills to review and critique a draft decision on health and biodiversity under the UN Convention on Biological Diversity (CBD). The team identified key points of relevance for Liberia and generated recommendations via a policy statement that was shared with Liberian government officials from the Environmental Protection Agency. This ultimately formed the basis of messages disseminated at the CBD Conference of the Parties and the inclusion of a clause recommending that countries conduct integrated health and biodiversity impact assessments.

PRACTICAL IMPLICATIONS

The PREDICT project changed the landscape for public health in Liberia. Our project established the first-of-its-kind wildlife surveillance team and successfully integrated into the existing public health infrastructure. In addition, our team played a major role in creating a new public health paradigm through the lens of One Health.

- The PREDICT project developed the country's first One Health surveillance team, which included wildlife biologists and field technicians able to safely sample wildlife and livestock for viral pathogens such as Ebola, and social scientists who conducted behavioral risk analysis and community outreach to reduce the risk of viral spillover from animals to people.
- Our team detected *Zaire ebolavirus* in a mine-dwelling bat in northern Liberia, the first time this virus had been identified in a bat in West Africa, providing important evidence that this virus continues to circulate in bats in the region.
- Following the discovery and announcement of *Zaire ebolavirus*, the PREDICT team worked closely with the National Public Health Institute, Liberia and the Ministry of Health to coordinate and implement a successful public risk communication campaign. As part of this campaign, our team developed and utilized an illustrated risk reduction and behavior change communication resource *Living Safely with Bats* for community education and provided the resource to the Ministry of Health to become part of their public risk communication toolkit.

SPECIAL FEATURES

PROACTIVE PATHOGEN DETECTION & RISK COMMUNICATION TO PROTECT COMMUNITIES LIVING CLOSELY WITH ANIMALS

The PREDICT project's support of the first wildlife veterinarian in Liberia led to critical workforce development where veterinary capacity is extremely limited. Ten field staff were trained on PREDICT project protocols on animal handling, sampling, and biosafety, and these trainings were deployed for use in extensive bat and rodent sampling efforts. The team was put to the test through intensive wildlife sampling resulting in the first ever detection of *Zaire ebolavirus* in a bat in West Africa.

In addition to continuing to shed light on the role of bats as the reservoir for high-consequence pathogens such as Ebola, the capacity of the trained wildlife disease investigation team was leveraged to provide research and management support for other zoonotic diseases, including rabies.

Liberia has embraced the One Health approach in light of the country's endemic and emerging zoonotic disease priorities, forming a One Health Secretariat with representation from human and animal health, forestry, and environmental agencies. This provides a strong platform to improve understanding of baseline risk, needs assessment for additional priority surveillance, and interventions to target high-risk activities. For example, Ebola is now included in the National Animal Health Surveillance Strategy developed in early 2019. The Ebola finding is also potentially relevant for the health and conservation of Liberia's wildlife, as other species are susceptible to Ebola virus.

Learn more here: **p2.predict.global/strengthening-health-security**

PUBLIC-PRIVATE PARTNERSHIPS TO PREVENT PANDEMICS

In 2016, when the PREDICT program initiated the Ebola Host Project in West Africa, EcoHealth Alliance (EHA), a PREDICT project global partner, was engaged in discussions with ArcelorMittal (AML), a global iron ore mining company present in Nimba County, northern Liberia. AML opened in Liberia in 2006 and represents one of the largest foreign investors in the country. They provide significant contributions to Liberia as an employer and also by building schools, hospitals, roads, and other infrastructure. AML approached our team about a colony of bats that was residing inside an unused exploratory mine shaft on its mining property. AML was planning to move the resident bats from the mine shaft into a new artificial cave they built within a forested area on their property.

Recognizing the potential for the bats to disperse, the Ministry of Health had asked AML to screen these bats for Ebola virus before moving them. The PREDICT team worked with AML to screen these and other bats on and around the property for Ebola and related viruses. In return, AML provided support to the PREDICT team, including providing housing and meals on their property during training and subsequent sampling activities, as well as on-site training facilities (e.g. conference rooms) and logistical support including security, equipment storage, and transportation during bat sampling activities. The bat samples tested negative for Ebola, and the bats were successfully translocated to the new artificial cave.

The PREDICT team returned to Nimba County several times throughout the 2016-2018 sampling period, occasionally working on AML property. In November 2018, a bat that had been captured and sampled on AML property tested positive for *Zaire ebolavirus* – the same virus responsible for the 2014 outbreak. The result was reported to the National Public Health Institute of Liberia (NPHIL) and the Ministry of Health. When the Government of Liberia made plans to publicly announce the finding, the Director General of NPHIL and the PREDICT team met with an AML executive in Monrovia to share the finding. ArcelorMittal reaffirmed their commitment to work in Liberia. Following the finding, the PREDICT team provided technical information to AML, which is being used to develop communications for their employees and to the communities around the mining concession.

INSIGHTS ON THE GEOGRAPHIC DISTRIBUTION OF MOLOSSID BATS IN WEST AFRICA

Through the Ebola Host Project, PREDICT teams in in Sierra Leone and later Guinea detected a new ebolavirus, Bombali virus in molossid bats. In recognition of the limited data available regarding bat distribution in the West Africa region, the Government of Guinea requested assistance identifying areas in the country and greater West Africa region at highest risk for viral spillover from bats. In response, we developed a spatial distribution model to identify areas that are ecologically suitable for habitation of Molossidae bats, such as *Mops condylurus* (Angolan free-tailed bat) and *Chaerephon pumilus* (little free-tailed bat), that were found to harbor Bombali virus.

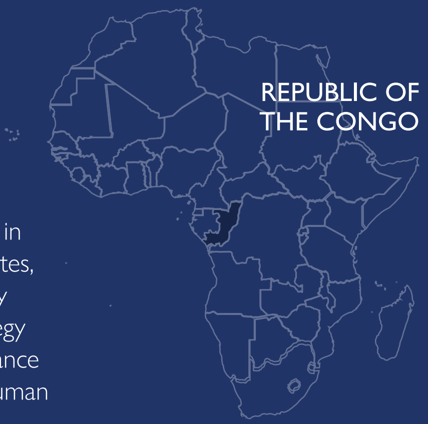
Using PREDICT project data, our model identified areas in the region that are suitable for habitation and where the bats may be present at higher densities, resulting in increased human contact and possibly higher viral spillover risk. Tools such as this model can assist the Governments of Guinea, Liberia, and Sierra Leone to better target wildlife surveillance and community-based risk reduction activities.

Learn more here: **bit.ly/3fH56Nw**

PREDICT

REPUBLIC OF CONGO

ONE HEALTH IN ACTION (2009-2020)



In the Republic of Congo, the PREDICT project began in 2009 with the general aim of reducing pandemic risk and promoting global health. Our objectives were to generate an evidence base that informs strategic investments in surveillance, detection, and response to zoonotic disease threats and to strengthen capacity of the health system to prevent and detect emerging infectious diseases. Public health capacity in the Republic of Congo (RoC) faces critical challenges, including a low density of health personnel and limited laboratory infrastructure. Wildlife-human contact interfaces are prevalent in many parts of the country, and the country has experienced outbreaks of zoonotic diseases, including an outbreak of monkeypox in 2017. However, RoC has recently made significant progress in zoonotic disease prevention and preparedness, using a One Health approach to promote improvements in public health capacity that benefit multiple sectors.

The PREDICT project put One Health in action and conducted wildlife surveillance in Ouesso within the Sangha Department, and in the Sibiti district and Zanaga districts within the Lékoumou Department. Fieldwork was conducted at high-risk animal-human interfaces such as artisanal gold mining, logging, road construction sites, and areas with ecotourism near Odzala-Kokoua National Park. As we worked to strengthen disease detection capacity in RoC, samples safely collected from wildlife were sent to the PREDICT project partner lab at the Institut National de Recherche Biomédicale/National Institute for Biomedical Research (INRB) in Kinshasa (Democratic Republic of the Congo) where they were tested for priority zoonotic diseases and other emerging viral threats.

Over 10 years, our team detected 70 unique viruses (56 new viruses and 13 known viruses) in samples collected from bats, non-human primates, and rodents. Our discoveries highlight the utility of the PREDICT project's virus detection strategy and underscore the need for proactive surveillance when confronting viral threats at the wildlife-human interface.

Another major focus in RoC from 2014 onwards were social and behavioral investigations to explore the risks of viral spillover and spread in the animal value chain. Our team performed in-depth, ethnographic interviews and focus groups with more than 100 individuals, targeting Brazzaville bushmeat market vendors and surrounding restaurants. By integrating this socio-anthropological approach with the evidence from surveillance and virus findings our team helped raised awareness of the risk of zoonotic diseases in people's daily activities.

The PREDICT project raised awareness of the importance of monitoring wildlife (rodents and bats), brought together various stakeholders involved in wildlife health, and fostered the creation of a One Health inter-ministerial group dedicated to examining emerging disease threats and developing contingency plans in case of an emergency outbreak. We collaborated with the Government of RoC to build wildlife surveillance capacity and strengthened multisectoral communications, sharing knowledge and informing surveillance and risk reduction strategies relevant for both the animal and public health sectors.

LOCAL PARTNERS

- Laboratoire National de Santé Publique
- Laboratoire de Diagnostique Vétérinqire de Brazzaville

>60

DEVELOPED the One Health Workforce by training more than 60 people in Republic of Congo.

>1.9K

OPERATIONALIZED One Health surveillance and sampled over 1.9K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

VIRUS FINDINGS

56 PREDICT

13 KNOWN

DETECTED 69 unique viruses in both animal and human populations.

CYNTHIA GOMA NKOUA

Country Coordinator, *Laboratoire National de Santé Publique*

"The contributions of PREDICT are incredibly valuable in terms of identifying weaknesses and strengths of the current biosafety and biosecurity procedures, sampling, zoonotic disease surveillance monitoring, reporting and mapping. This experience has confirmed my desire to improve the technical and managerial capacities of our institution."

HILARION MOUKALA NDOLO

Behavioral Risk Survey Investigator, *Independent Consultant*

"I was hired by PREDICT to perform ethnographic interviews, focus groups and questionnaires in the communities and bushmeat markets in Brazzaville. In these studies, we discovered there are those who don't understand the concept of protected animal species, nor that handling animal species presents multiple risks of contamination with zoonotic diseases agents. I want to find ways to better educate communities and raise awareness about wildlife conservation."

ACHIEVEMENTS

- Published evidence-based animal surveillance recommendations for a series of human Ebola virus outbreak investigations in Africa
- Worked with the National Public Health laboratory (Laboratoire National de Santé Publique, LNSP) to strengthen disease detection capacity for emerging viral threats and priority zoonoses
- Advocated for the establishment of an EPT Consortium as part of the national One Health platform through the establishment of an International Health Regulations Committee
- Enhanced disease surveillance capacity and coordination among animal and public health officials to ensure communication across health sectors
- Contributed to development of the Ebola contingency plan and operationalization of the One Health framework

ONE HEALTH SURVEILLANCE

From 2014 onwards, the PREDICT project in RoC sampled bats and rodents in Sibiti and the Brazzaville area, two sites with high priority animal-human interfaces for One Health surveillance. Brazzaville is the capital city and one of the major urban areas in country. In Brazzaville residents engage in small-scale agriculture, but a large portion of the local diet is derived from meat, from both domesticated animals and hunted or purchased bushmeat from local wildlife markets (either live for slaughter, fresh, or smoked). These markets were targeted for behavioral risk investigations to explore the dynamics of the markets and learn more about the potential social and biological dimensions of disease transmission. In the markets, individuals in many high-risk occupations (hunters, vendors, and consumers) are directly exposed to potential pathogens in domestic animals, live wildlife, and bushmeat.

Sibiti, located between Brazzaville and Pointe-Noire, has a population of >20,000 people and straddles the forest and plains of the Valley of the Niari. Sibiti, along with surrounding towns is a central attraction and one of the largest suppliers of bushmeat in the area for the larger cities of Dolisie, Nkayi, Pointe-Noire, and Brazzaville. Economically, agriculture, forestry, mining, and hunting are main contributors to livelihoods, though two local companies in the extractive industrial sector employ a large number of workers. We conducted surveillance in Sibiti as the close proximity of wildlife to humans, the presence of the mining and forestry industries, restaurants selling wildlife, and markets that trade fresh and smoked bushmeat present unique interfaces for viral spillover from wildlife.

VIRUS TABLE (2014-2019)

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	TOTAL POSITIVE INDIVIDUALS
Coronavirus	PREDICT_CoV-30	Franquet's Epauletted Fruit Bat, Woermann's Fruit Bat	Zanaga district	2
	Kenya bat coronavirus/ BtKY56/BtKY55	Angolan Free-Tailed Bat, Peter's Dwarf Epauletted Fruit Bat, Unidentified Myonycteris Fruit Bat	Brazzaville	9
Total				**11**

VIRUS DETECTION

From 2014 onwards, the PREDICT project's strategy for virus detection focused on screening samples using broadly reactive consensus PCR (cPCR) for five priority virus groups: coronaviruses, filoviruses, flaviviruses, paramyxoviruses, and influenza viruses. Prior to 2014, we screened samples for a broader range of virus groups such as adenoviruses, bunyaviruses, and herpesviruses (see Further Reading below). Positive samples detected using these assays were sequenced to identify the viruses and compare their relationship to known pathogens, and viruses were prioritized for further characterization. This approach allows for detection of both known and novel viruses and improves our understanding of the presence and diversity of viruses, as well as potential pathogens, in humans and animals.

In RoC, as we worked to strengthen disease detection capability at the National Public Health Laboratory (Laboratoire National de Santé Publique, LNSP), wildlife samples collected from surveillance sites were transported to the PREDICT project's partner lab, INRB in neighboring DR Congo.

VIRUS FINDINGS IN WILDLIFE

Two coronaviruses (one known and one new coronavirus) were detected in bats from samples collected between 2014 and 2016.

A strain of the known Kenya bat coronavirus BtKY56/BtKY55 was detected in 9 bats at the Brazzaville site. This virus was detected in multiple species of bats including Peter's dwarf epauletted fruit bats (*Micropteropus pusillus*), Angolan free-tailed bats (*Mops condylurus*), and unidentified fruit bats belonging to the *Myonycteris* genus.

Additionally, a new betacoronavirus, PREDICT_CoV-30, was detected in two bats: a Franquet's epauletted fruit bat (*Epomops franqueti*) and a Woermann's fruit bat (*Megaloglossus woermanni*) at the Zanaga district site. While Betacoronaviruses include viruses of public health significance, such as SARS and MERS, currently, there is no evidence to suggest that this virus poses a threat to human health.

INVESTIGATING BEHAVIORAL RISKS IN BUSHMEAT MARKETS AND THE WILDLIFE VALUE CHAIN

The PREDICT project's behavioral risk team had the unique opportunity to investigate social and behavioral dynamics in a large bushmeat market. Working in 12 sites throughout Brazzaville and the Pool, our team used in-depth one-on-one interviews and focus group discussions to engage with the diverse actors in the value chain: hunters, suppliers and middlemen, vendors, consumers, and employees of adjacent shops and businesses.

UNDERSTANDING THE BAT-HUMAN INTERFACE

Two coronaviruses (one known and one new coronavirus) were detected in bats from samples collected between 2014 and 2016. While these semi-structured investigations covered a wide range of wildlife taxa involved in the bushmeat trade, the team was particularly interested in learning about the ways in which bats were handled and consumed, and how bushmeat contributed to individuals' livelihoods. The interviews, along with our team's observations, made it clear that bats were a visible staple in the marketplace. Bats were described as being available for purchase either freshly killed and ready to be butchered at home, or alive and available to be slaughtered on demand in the market. Smoked bushmeat was also a recurring preference. According to respondents who reported slaughtering bats (putting them into direct contact with the viscera), bats were seen as having little that could not be consumed. In addition, when asked about Ebolaviruses and the risks associated with bat consumption, an adult female shop owner shared her perception, "since we saw nobody die of it, we keep eating them." Other respondents shared that though they did not sell nor eat bats, this did not necessarily mean that they did not handle the animals, as they would handle the animals during the course of preparing meals for other household members.

Hunters also spoke at length about the techniques they used to capture the animals. A commonly described method was using nets in trees to live capture bats – a method that necessitated precaution around sharp teeth and claws. Several respondents described having been bitten or knowing others who had been bitten by bats. Bat teeth were seen as particularly nasty, as illustrated by the following comments.

> "I just wanted to point out that really hunting bats is something that helps us a lot and with this hunt we provide for our families."
>
> –Adult male trapper

> "Well we buy [them] at the market. The prices of bats vary according to their sizes. They are often in the cages.When we buy them, we come back with them home."
>
> –Female bushmeat consumer

> "Yes, I kill them myself. [...] The bat's skin is hard, so it takes a lot of time to [cook]. When it is cooked, I can now put the sauce, or I only make a simple broth."
>
> –Male bushmeat consumer

CONTRIBUTIONS TO LIVELIHOODS & FOOD SECURITY

Income generated from bat sales varied based on the size of the animal and ranged from 500 to 1,500 francs (~$0.25-0.75 USD). Bat hunting, particularly during mango season, could yield substantial income for a family, and working with bats was perceived as the only way to make ends meet for some households.

Other respondents highlighted the importance of bats to household food security and nutrition describing the purchase, slaughter, and preparing of bat meat.

> "When the bat really bites you, it is hard to remove it because its teeth are like claws. It can stay there even up to an hour."
>
> –Male bushmeat hunter

> "You have to be very careful because they have sharp teeth, you have to know the techniques otherwise you can lose your fingers."
>
> –Male bushmeat consumer

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

When the PREDICT project's risk reduction and behavioral change communication resource *Living Safely with Bats* was translated into French "Vivre en toute sécurite avec les chauves-souris," the team in RoC adapted the resource as an easy-to-carry, small format tool to use in the field. Outreach events were organized in the four Brazzaville markets where the PREDICT behavioral work was carried out by our behavior risk team. Street vendors of bats, bushmeat vendors, and heads of each market were provided with the bat book and were engaged in a dialogue balancing health and conservation goals to raise awareness about the risks of disease transmission, along with the benefits of bats to our ecosystems.

CAPACITY STRENGTHENING

ESTABLISHING NATIONAL ONE HEALTH PLATFORMS

Through the PREDICT project our team pioneered advocacy and promotion of One Health in RoC. We helped to successfully establish a multi-sectoral One Health Consortium involving: Ministry of Defense Ministry of Agriculture, Ministry of Forestry and Wildlife, Ministry of Environment, Ministry of Scientific Research, Ministry of Finance, Homeland Ministry, World Health Organization (WHO), and the Food and Agriculture Organization of the United Nations (FAO). Our team worked the Ministry of Health and WHO to establish the Consortium and to operationalize One Health at the national level through the establishment of an International Health Regulations committee, which in RoC acts both as a One Health Consortium as well as an inter-sectoral/multi-sectoral committee for preparedness and response to disease outbreaks. The body is chaired by the Director General of Epidemiology and Disease Control and Response (DGELM) and is one of the major success stories of the PREDICT project's investment. Under this multi-sectoral framework the PREDICT RoC team supported the investigation of the Monkeypox outbreak in the North zone of the country in 2017, effectively moving One Health from policy to action for disease surveillance and control.

STRENGTHENING THE NATIONAL LABORATORY SYSTEM

At the national public health laboratory (LNSP), the PREDICT project invested in laboratory infrastructure with support from the NIH. Equipment and supplies were procured in 2017, and the PREDICT team supported staff at LNSP to oversee quality control of the equipment and implement disease detection protocols. Funds were allocated to outfit the laboratory with a sustainable power system provided by Africa Solaire, which facilitated operation of all essential lab equipment and ensured maintenance of cold chain for safe sample storage.

In addition, the PREDICT project provided unique opportunities for One Health training and collaboration, and engaged ministry staff, as well as staff from the national laboratory system, LNSP and the national veterinary diagnostic laboratory (Laboratoire de Diagnostic Vétérinaire de Brazzaville, LDVB) in capacity strengthening efforts. The PREDICT project offered one of the most comprehensive training opportunities in disease detection. Staff in the national laboratory system were trained to meet the needs of the Ministries of Health, Agriculture and Forestry and to support the efforts of the Ministry of Defense. These efforts greatly increased the country's capacity for surveillance, diagnostics, and biosafety. Improvements in training and laboratory facilities were put to the test during the chikungunya outbreak in 2019, demonstrating the benefits of a prepared workforce and well-equipped laboratory for detection and response to health emergencies. There is continued interest from the Ministry of Agriculture for training staff and analyzing samples and fostering collaboration across sectors. In addition, the laboratory conducts tests to investigate wildlife mortalities, which are now recognized as a possible public health threat in the country, given earlier discoveries that ape deaths can precede human cases of Ebola virus, and that sustained investments in wildlife surveillance can provide "early warning" for triggering potential public health interventions.

PRACTICAL IMPLICATIONS

- The history of zoonotic disease outbreaks with wildlife origins in RoC and the diversity of virus findings identified in rodents, bats, and non-human primates over 10 years of the PREDICT project in this country highlight the continued need for vigilance in wildlife surveillance for emerging viral threats.
- PREDICT project investment at LNSP empowered the national laboratory system for success in detection and response to disease threats; these investments in facilities and human resources are critical to sustain for achieving national health security.
- PREDICT project personnel along with LNSP staff and other stakeholders were engaged in several outbreak preparedness, risk communication, and disease investigation collaborations. Multi-sectoral partnerships that move One Health from policy to action are crucial for addressing outbreaks at their source before they turn into epidemics.
- In-depth behavioral risk investigations were conducted in multiple bushmeat markets and restaurants in Brazzaville. These markets constitute an important animal value chain and disease risk interface, and our data provide clear insights into the complexities of the bushmeat trade and health risks layered with the importance of bushmeat to livelihoods and food security. These insights are invaluable for informing interventions designed to reduce spillover risk while simultaneously respecting livelihoods, cultural norms, and wildlife conservation objectives.

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PREDICT RWANDA

ONE HEALTH IN ACTION (2009-2020)

Rwanda is a densely populated nation in East Africa – a part of sub-Saharan Africa that is a geographic "hotspot" for emerging infectious disease. Subsistence agriculture dominates as the primary source of food and livelihoods. At the same time, Rwanda enjoys a high level of wildlife biodiversity. The Government of Rwanda prioritizes conservation through careful and intense management of protected areas that comprise about 10% of the country's total landscape, including Volcanoes National Park, the home of the endangered mountain gorillas, and Nyungwe National Park, containing 12 endemic species of non-human primate.

High biodiversity combined with high human population density creates numerous opportunities for wildlife-human interaction. In particular, mountain gorilla tourism brings thousands of people from around the world and from local communities into close contact with human-habituated mountain gorillas every day. Because gorillas share 98.5% of their genome with humans, transmission of infectious disease remains an ever-present threat to both the gorillas and people. Furthermore, wild bats and rodents in Rwanda are cosmopolitan in nature, often residing in urban centers and in human dwellings.

From 2009-2019, our team conducted field and laboratory surveillance for viral pathogens circulating in wildlife in Rwanda. In collaboration with Rwanda's Ministry of Agriculture and the Rwanda Development Board, PREDICT established the first wildlife laboratory in Rwanda, and safely screened wildlife and human samples for up to 14 virus groups of concern to human health. Over 10 years, more than 2,500 wild animals (wild primates, bats and rodents) were safely and humanely captured and sampled, because these taxa have a high rate of contact with people in Rwanda, and have proven to be reservoirs for previously-known zoonotic pathogens.

As well, PREDICT Rwanda screened 400 consenting febrile patients presenting to rural health centers neighboring Volcanoes National Park. Our team conducted questionnaires and collected biological samples to better understand the level and type of contact people have with wildlife in the region. Wildlife samples were first processed at the Wildlife Virology Laboratory, and human samples were processed at the Rwanda Biomedical Center (RBC). All samples were analyzed for the presence of virus either at RBC's National Reference Laboratory, where staff were trained on PREDICT laboratory testing protocols, or at the University of California, Davis One Health Diagnostic Laboratory.

Through analysis of project data and findings, PREDICT was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

LOCAL PARTNERS

- Rwanda Development Board/Tourism and Conservation Department
- Rwanda Agriculture Board
- Rwanda Biomedical Center/National Reference Laboratory
- University of Rwanda

**Photos in this report credited to Gorilla Doctors unless stated otherwise*


>200

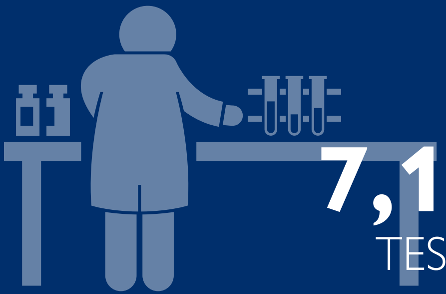
DEVELOPED the One Health Workforce by training more than 200 people in Rwanda.


>2.9K

OPERATIONALIZED One Health surveillance and sampled over 2.9K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Rwanda Agriculture Board Wildlife Virology Laboratory
- National Reference Lab/Rwanda Biomedical Center


7,111
TESTS


21 PREDICT

19 KNOWN

DETECTED 40 unique viruses in both animal and human populations.

JULIUS NZIZA

Country Coordinator, *Gorilla Doctors*

"Implementing PREDICT in Rwanda has changed my professional life forever, and for the better. PREDICT work has been exciting in all aspects, from conducting field surveillance to engaging in laboratory work and establishing collaborations."

JEAN CLAUDE TUMUSHIME

Project Veterinarian, *Gorilla Doctors*

"Working for PREDICT has been a life-changing experience for my professional career in many ways. I feel proud for having been part of a team that is at the forefront of helping public health and conservation sectors design interventions that can be used to deter the next pandemic threat."

ACHIEVEMENTS

- Established the first wildlife virus testing laboratory in Rwanda. The laboratory is located in the Rwanda Agriculture Board's livestock health facility, thereby enhancing Rwanda's commitment to One Health and its focus on building One Health platforms for coordinated human and animal health surveillance and threat mitigation.
- Detected 21 novel viruses and 19 documented known viruses at critical wildlife-human interfaces in Rwanda. This information was shared with government partners to benefit both wildlife conservation and public health.
- Detected several known and novel coronaviruses in bats – a family of viruses known for causing recent emerging infectious diseases like SARS, MERS, and the current COVID-19 pandemic.
- Documented the "reverse" transmission of zoonotic pathogens from people to wildlife (gorillas).
- Interviewed 400 people to better understand the social and behavioral factors associated with zoonotic disease transmission risk.

(Opposite) PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

ONE HEALTH SURVEILLANCE

Rwanda is a densely populated nation in sub-Saharan East Africa that is a geographic "hotspot" for emerging infectious disease. Subsistence agriculture dominates as the primary source of food and livelihoods. At the same time, Rwanda enjoys a high level of wildlife biodiversity, and the Government of Rwanda prioritizes conservation through careful and intense management of protected areas that comprise 10% of the country's total landscape, including Volcanoes National Park, the home of endangered mountain gorillas. High biodiversity combined with human population density creates numerous opportunities for wildlife-human interaction. In particular, mountain gorilla tourism brings thousands of people from around the world and from local communities into close contact with human-habituated gorillas every day. Because gorillas share 98.5% of their genome with humans, human-borne infectious disease remains an ever-present threat to this population. Furthermore, wild bats and rodents in Rwanda are cosmopolitan in nature, often residing in urban centers and in human dwellings, and bat roosting caves are gaining in popularity as a wildlife tourism destination.

In Rwanda, PREDICT has built an evidence base for priority zoonoses and emerging diseases at the wildlife-human interface. PREDICT focused its work in local communities surrounding Volcanoes National Park where there is a high-risk for wildlife-human contact. And working closely with government partners, PREDICT helped build capacity for recognition of zoonotic diseases and potential transmission pathways, supporting Rwanda's One Health workforce to prepare for emerging infectious disease prevention and control.

WILDLIFE SURVEILLANCE

In Rwanda, Volcanoes National Park and its surrounding communities is a site where people and wildlife come into daily close contact through wildlife tourism and subsistence farming. The Virunga Massif region of Central-East Africa is among the most densely human-populated areas on the continent, and is further unique as a One Health site in that thousands of people come from around the world every year for the opportunity to view human-habituated mountain gorillas daily. Subsistence farming directly abuts the park boundary, and wildlife (e.g. gorillas, other non-human primates, buffalo) exit the park to feed in crop lands.

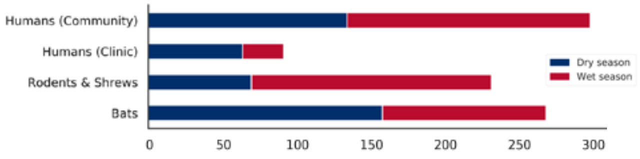
To identify viruses circulating in wildlife that have the potential to infect people, PREDICT surveillance efforts focused primarily on nonhuman primates, bats and rodents entering or living in human dwellings and accessing agricultural grounds and crops in communities adjacent to Volcanoes National Park. In addition, bats roosting in caves utilized for tourism were a focus for surveillance.

A total of 430 wild bats (insectivorous and frugivorous) were humanely live-captured in mist nets set up in villages and near roosting caves, and 286 wild rodents were humanely live-trapped in and around human dwellings and croplands. Non-human primates (n=25) were sampled opportunistically when clinical interventions were required to treat injured or ill human-habituated mountain gorillas, or when other primates (e.g. golden monkeys) exited the park and were encountered in public buildings (e.g. schools), dwellings, or crop lands.

HUMAN SURVEILLANCE

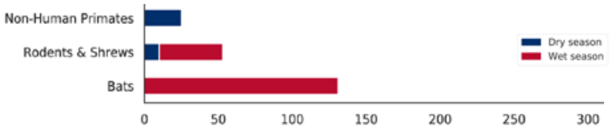
Concurrently, over a 7-month period between December 2016 and July 2017, people living in the communities bordering Volcanoes National Park where wildlife sampling was conducted, and who presented to local community health centers with fevers of unknown origin, were included in the PREDICT One Health surveillance effort. Community health centers in which patients were sampled were located in the villages of Kinigi, Shingiro, and Bisate, and at Ruhengeri Regional Hospital in Musanze (Northern Province), all of which serve a wide-ranging community outside of the villages where the health centers are located. Patients who gave their consent for enrollment in the surveillance program provided biological samples (blood, mucosal swabs) and answered questionnaires about their demographics, livelihoods, and contact with animals (domestic and wild). Children (under the age of 18) were only enrolled with parent or guardian consent at the health centers. A total of 400 people were sampled and surveyed, including 123 adult men, 207 adult women, and 70 children.

MUSANZE REGION



Numbers of individuals sampled by taxa

NYUNGWE NATIONAL PARK REGION



Numbers of individuals sampled by taxa

VIRUS DETECTION

A subset of samples from wildlife were first processed (nucleic acid extracted) at the Wildlife Virology Laboratory at the Rwanda Agriculture Board's Rubirizi facility (Kigali, Rwanda), and then both extracted and raw (unextracted) wildlife samples were shipped to the One Health Institute Diagnostic Laboratory at the University of California, Davis (USA), where they were tested for coronaviruses, paramyxoviruses, filoviruses, flaviviruses, and influenza viruses using a broad-based consensus PCR platform. All human samples were tested at the National Reference Laboratory at the Rwanda Biomedical Center (Kigali, Rwanda), also for coronaviruses, paramyxoviruses, filoviruses, flaviviruses, and influenza viruses, using the same broad-based consensus PCR platforms. In all cases, initial results from PCR screening were confirmed via genomic sequencing in the USA. Test results were shared with the Government of Rwanda through the appropriate ministries and departments and can be viewed at **www.data.predict.global**

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	Coronavirus 229E (Human strain)	Human	Bisate Health Center (Musanze), Ruhengeri Hospital (Musanze), Shingiro Health Center (Musanze)	6	0	6
	Human coronavirus HKU1	Human	Shingiro Health Center (Musanze)	2	0	2
	PREDICT_CoV-44	Angolan Fruit Bat, Sundevall's Roundleaf Bat	Nyamagabe	7	7	0
	PREDICT_CoV-77	Angolan Fruit Bat	Nyamagabe	1	1	0
	PREDICT_CoV-97	Egyptian Fruit Bat	Musanze	2	0	2
	Bat coronavirus HKU9	Egyptian Fruit Bat	Musanze	1	0	1
	Chaerephon bat coronavirus/Kenya/KY22/2006	Giant Mastiff Bat	Musanze	5	1	4
	Coronavirus 229E (Bat strain)	Angolan Fruit Bat, Sundevall's Roundleaf Bat	Nyamagabe	2	2	0
	Eidolon bat coronavirus	Straw-Coloured Fruit Bat	Kigali, Nyamagabe	13	1	12
	Kenya bat coronavirus/BtKY33/2006	Unidentified Miniopterus Bat	Nyamagabe	1	1	0
	PREDICT_CoV-94	Black Rat	Musanze	1	1	0
Paramyxovirus	Human parainfluenzavirus 1	Human	Kinigi Health Center (Musanze)	1	0	1
	Mumps virus	Human	Kinigi Health Center (Musanze)	1	1	0
	PREDICT_PMV-56	Straw-Coloured Fruit Bat	Kigali	1	0	1
	PREDICT_PMV-179	Egyptian Fruit Bat	Musanze	1	1	0
Influenza virus	Influenza A	Human	Kinigi Health Center (Musanze)	5	0	5
	Influenza B	Human	Bisate Health Center (Musanze), Kinigi Health Center (Musanze)	3	2	1
Total				**53**	**18**	**35**

VIRUSES DETECTED IN HUMANS

We detected viral RNA in 5% (18/392) of human patients sampled and tested for priority virus groups. Eight patients were positive for coronaviruses, including a strain of the Human coronavirus HKU1 (n=2 patients) and the known alphacoronavirus Human Coronavirus 229E (n=6 patients): both of these coronaviruses are known to cause respiratory illness in people. We detected influenza viruses in eight patients, including influenza A in five individuals, as well as influenza B in three children. We also detected known paramyxoviruses, including mumps (n=1 patient) and Human parainfluenzavirus 1 in one adult patient.

VIRUSES DETECTED IN WILDLIFE

We tested 430 bats, 286 rodents, and 25 non-human primates. We detected viral RNA from five known and three novel coronaviruses in 31 individual bats (*Eidolon helvum*, n=13; *Hipposideros caffer*, n=4; *Lissonycteris angolensis*, n=4; *Otomops martiensseni*, n=5; *Rousettus aegyptiacus*, n=4); and *Miniopterus* sp., n=1). The novel bat coronaviruses PREDICT_CoV-77 and PREDICT_CoV-44 are both in the *Betacoronavirus* genus, which is known to include viruses of potential public health significant, such as SARS, MERS, and SARS CoV-2, the cause of COVID-19. However, these new viruses detected in bats in Rwanda are not closely related to SARS or MERS. Therefore, at this time, there is no evidence to suggest these viruses pose a threat to human health. As well, the novel bat coronavirus, PREDICT_CoV-97 is in the alphacoronavirus family, and has also been found in bats in Cameroon and Tanzania. There is no evidence at this time to suggest that this virus poses a risk to human health.

For most of the known bat coronaviruses detected in Rwanda bats – coronavirus 229E (bat strain), Chaerephon bat coronavirus/Kenya/KY22/2006, Kenya bat coronavirus BtKY33/2006, Eidolon bat coronavirus, and bat coronavirus HKU9 – there is no evidence to suggest that these viruses pose a threat to human health.

A novel paramyxovirus, called PREDICT_PMV-56, was detected in one individual bat (*Eidolon helvum*): this virus is in the *Rubulavirus* genus, and there is no evidence at this time to suggest it poses a threat to human health. Three individual bats were co-infected with multiple viruses: two bats (*Hipposideros caffer* and *Lissonycteris angolensis*) were each co-infected with coronavirus 229E (bat strain) and PREDICT_CoV-44, and one bat (*Eidolon helvum*) was co-infected with a known strain of Eidolon bat coronavirus and a novel paramyxovirus (PREDICT_PMV-56).

A novel coronavirus in the *Alphacoronavirus* genus (PREDICT_CoV-94) was detected in one rodent (*Rattus* sp.). There is no evidence at this time to suggest this virus poses a threat to human health.

We did not detect virus in any of the 25 non-human primates tested.

PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

EPIDEMIOLOGIC & BEHAVIORAL RISK

Human surveillance conducted at Ruhengeri Hospital and the health centers in Kinigi, Bisate, and Shingiro included both biological sampling, as described above, as well as qualitative and quantitative behavioral data collection in patients presenting with fevers of unknown origin. From December 2016 to July 2017, 400 patients participated in clinic-based surveillance and were administered demographic and behavioral questionnaires for syndromic surveillance purposes.

EDUCATION & DISEASE AWARENESS

Of the 355 patients over the age of 12 that reported illness in the previous year, 34% of individuals stated that they did not know the cause of their illness or sickness. Of those, 20% had no formal education, 55% completed primary school, and 25% completed secondary school. Twenty-five percent (25%) of respondents said the cause of their illness was contact with sick people, and of those, 10% had no schooling, and 44% and 46% had completed primary and secondary school, respectively. Bad food or water as a reason for illness was the response offered by 24% of those surveyed, of which 17% had no schooling, while 55% had completed primary school, and 28% secondary school. Other responses included climate change (6%) and bad spirits or witchcraft (2%) as causes of illness. Approximately 1% stated that contact with wild and domestic animals could be a cause of their illness.

CLINIC-BASED SURVEILLANCE PARTICIPANT CHARACTERISTICS (n=400)

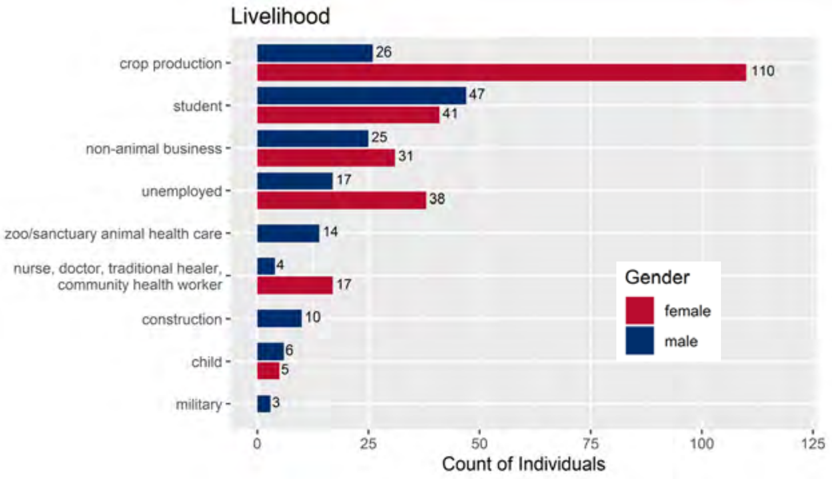
SITES	FEMALE	MALE
Bisate Health Center	61	39
Kinigi Health Center	60	40
Ruhengeri Hospital	54	46
Shingiro Health Center	70	30

LIVELIHOODS

A majority of the 245 females interviewed were engaged in crop production (45%) for their livelihoods; 17% were students, 16% were unemployed, and 13% were engaged in non-animal-related businesses. Of 155 males interviewed, most were students (30%), engaged in crop production (17%), or in non-animal related businesses (16%).

DEMOGRAPHICS OF HUMANS INTERVIEWED IN MUSANZE (n=400)

	NUMBER OF INDIVIDUALS
GENDER	
FEMALE	245 (61.2%)
MALE	155 (38.8%)
AGE GROUP	
ADULT (>18 YEARS OLD)	330 (82.5%)
CHILD	70 (17.5%)
AGE	
MEAN (SD)	30.4 (16.0)
MEDIAN [MIN,MAX]	28.0 [2.10, 97.0]



SYMPTOMS

Across all enrolled patients, the average days with fever prior to presentation at the clinic was 4.2 days. The most common symptoms in addition to fever were: headache (86%), cough (76%), cold (52%), sore throat (48%), and chills (46%). Females were more likely to report fever with headache (odds ratio (OR) = 2.06) and fever with cough (OR = 1.89) than males. There were no statistical differences between males and females for other symptoms.

REPORTED CONTACT WITH WILD ANIMALS

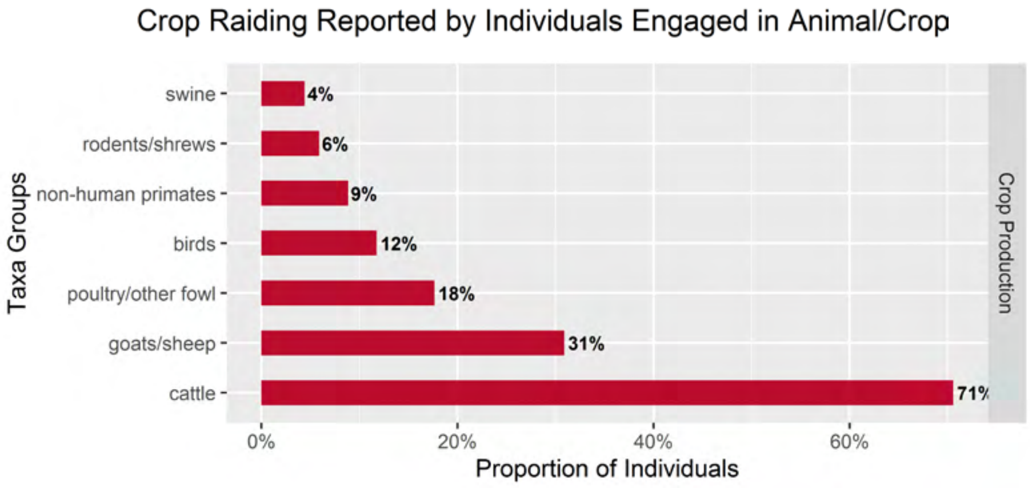
Contact with wild animals was primarily reported as keeping these animals as pets and included groups such as rodents/shrews, bats, birds, non-human primates, carnivores, and pangolins. A majority of the 245 females interviewed were engaged in crop production (45%) for their livelihoods; (17%) were students, (16%) were unemployed, and (13%) were engaged in non-animal-related businesses. Of 155 males interviewed, most were students (30%), engaged in crop production (17%), or in non-animal related businesses (16%).

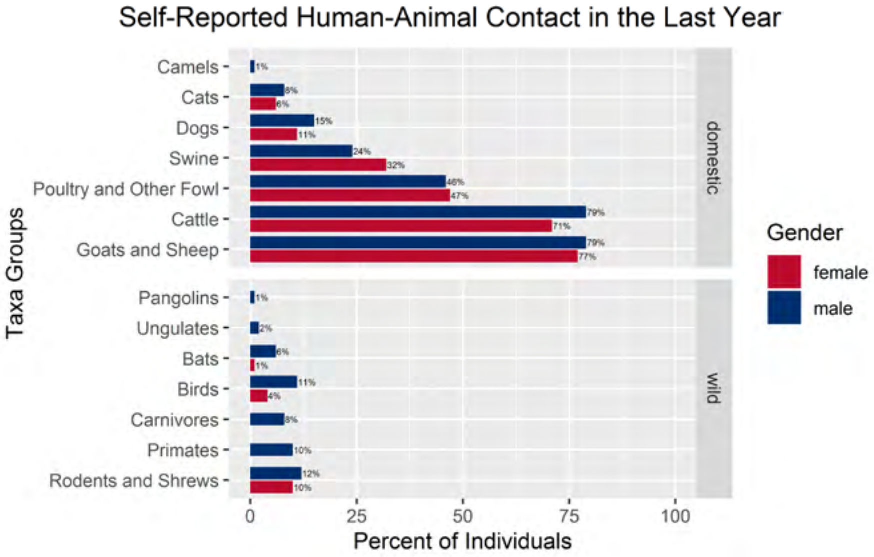
REPORTED CONTACT WITH DOMESTIC ANIMALS

Both males and females reported contact with a variety of domestic animals, including goats and sheep, cattle, poultry and other fowl, and pigs. For both goats, sheep and cattle, the most common forms of contact were cooking or handling, eating raw or uncooked meat, and raising and handling live animals. More males (15%) than females (1.2%) reported slaughtering goats and sheep. Similarly, only males reported slaughtering cattle (6% of male respondents). Both genders reported poultry and other fowl to be living in their home, and finding poultry feces in and around their food supply.

LIVELIHOOD-SPECIFIC ANIMAL CONTACT

Of the 136 patients who engaged in crop production for their livelihoods, all reported that domestic and wild animals raided food supplies or destroyed crops. Cattle were most commonly reported as crop-raiders (71% of respondents), followed by goats and sheep, poultry, and wild birds. Individuals also reported crop-raiding by other wildlife taxa, including non-human primates (9%) and rodents (6%).


Crop Raiding Reported by Individuals Engaged in Animal/Crop
Taxa Groups
swine
4%
rodents/shrews
6%
non-human primates
9%
birds
12%
poultry/other fowl
18%
goats/sheep
31%
cattle
71%
0%
20%
40%
60%
Proportion of Individuals
Crop Production


Self-Reported Human-Animal Contact in the Last Year
Taxa Groups
Camels
1%
Cats
8%
6%
Dogs
15%
11%
Swine
24%
32%
Poultry and Other Fowl
46%
47%
Cattle
79%
71%
Goats and Sheep
79%
77%
domestic
Pangolins
1%
Ungulates
2%
Bats
6%
1%
Birds
11%
4%
Carnivores
8%
Primates
10%
Rodents and Shrews
12%
10%
wild
0
25
50
75
100
Percent of Individuals
Gender
female
male

RISK COMMUNICATION

Prior to and during field surveillance, PREDICT conducted community outreach in all the district sectors where it implemented its activities. Outreach activities centered on interactions with local leaders about the project and its purpose. At the same time community members residing near the surveillance sites were sensitized about the different surveillance activities that were being conducted by PREDICT in that area, in order to foster harmony and good cooperation among project personnel, local leaders, and community members. Using the PREDICT booklet *Living Safely with Bats*, PREDICT initiated and is continuing to conduct community outreach to promote behavioral change for risk reduction in the local communities living near Musanze Caves where PREDICT conducted surveillance of roosting bats for viral pathogens.

As well, PREDICT participated in what fortunately turned out to be a "false alarm" response during an avian influenza (AI) alert in 2017. Due to the reported AI outbreak in Uganda, the Rwanda Agriculture Board (RAB) conducted massive sensitization about the disease, which led to many communities reporting on potential disease occurrence in birds at the local level. PREDICT joined RAB in responding to a report of dead wild birds in Bugarama, Rusizi District, bringing bird sampling equipment and supplies (no dead birds were observed at the site). PREDICT worked with government partners to continue sensitization about the disease in the Western and Northern Provinces via radio address.

STRENGTHENING CAPACITY

PREDICT Rwanda established the first wildlife virus testing laboratory in Rwanda and collected wildlife samples from major taxa for infectious diseases surveillance. The laboratory is located in the Rwanda Agriculture Board's livestock health facility, thereby enhancing Rwanda's commitment to One Health and its focus on building One Health platforms for coordinated human and animal health surveillance and threat mitigation.

PREDICT Rwanda discovered novel viruses and documented known viruses at critical wildlife-human interfaces in Rwanda. This information was shared with government partners to benefit both wildlife conservation and public health aspects.

Having established a Biosafety Level 2+ wildlife virology laboratory, PREDICT trained both wildlife and human laboratory personnel on consensus PCR techniques to analyze samples for novel and known viral pathogens. In addition, the team was trained on laboratory biosafety and contamination control by University of California, Davis research laboratory technicians.

As well, using PREDICT modules, PREDICT trained in-service personnel from the Government of Rwanda's animal and human health sectors and University of Rwanda veterinary students on Biosafety, Personal Protective Equipment use, Laboratory Biosafety, and Animal Capture techniques for bats, non-human primates, and rodents.

Lastly, PREDICT Rwanda was uniquely positioned to help develop new tools for non-invasive sample collection from wild non-human primates, with application to Rwanda's mountain gorilla populations an opportunity to optimize and show proof of concept of these tools.

PHOTO: SKYLER BISHOP FOR GORILLA DOCTORS

PRACTICAL IMPLICATIONS

There is now ample evidence for wildlife as potential reservoirs of zoonotic viral pathogens in Rwanda: in particular, PREDICT detected several known and novel coronaviruses in bats – a family of viruses known for causing global pandemics like SARS and MERS, and the current COVID-19 pandemic.

As well, PREDICT Rwanda documented the "reverse" transmission of zoonotic pathogens from people to wildlife (gorillas).

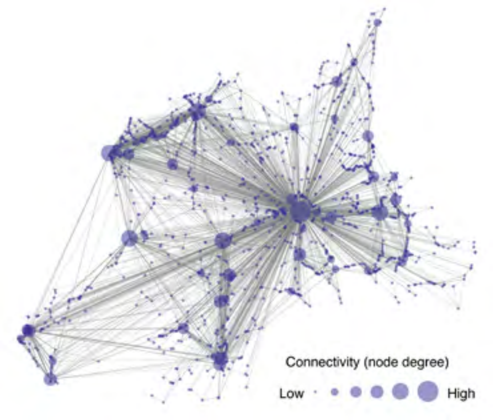
Behavioral surveillance in people working in and living around Volcanoes National Park revealed a high level of daily contact with wildlife, and therefore the potential for virus sharing, or "spillover."

In a country with one of the most well-developed wildlife tourism operations on the continent, which brings people from all over the world into close contact with wildlife, the threat for bidirectional viral spillover is real.

It is therefore recommended that wildlife pathogen surveillance and characterization, comprehensive virus screening in febrile patients, and studies to better understand the ecological and behavioral drivers of viral spillover, be continued.

SPECIAL FEATURE

SATELLITE ENHANCED EPIDEMIC & DISEASE



PREDICT assisted scientists at UC Davis to develop an outbreak modeling tool that utilized human population and satellite-based geospatial data to simulate disease spread. The network model depicts human connectivity and mobility, commute rates, and roads connecting urban centers and rural settlements. In simulations, the tool was able to accurately predict the epidemic size and geographic spread of the 2009 H1N1 influenza A pandemic in Rwanda.

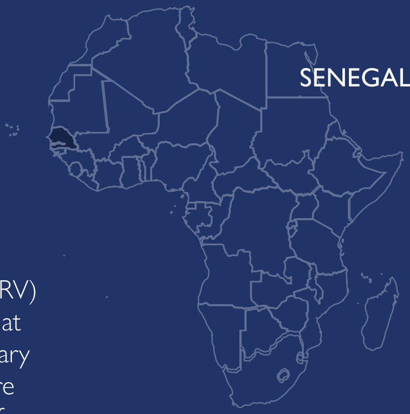
This work was published as an Emerging Disease Insights for USAID and is available electronically:

bit.ly/3aVTJPi

For more information view the interactive report at **p2.predict.global**

PREDICT
SENEGAL

ONE HEALTH IN ACTION (2017-2020)



One Health surveillance in Senegal

With numerous outbreaks occurring in the region, such as the 2014 Ebola Outbreak, and Lassa fever outbreaks in Nigeria and Sierra Leone, Senegal has been actively working to enhance its capacity for surveillance, and for detection of and response to emerging and re-emerging infectious diseases, 75% of which are of animal origin. The Senegalese government has undertaken several innovative actions in this direction, most notably adopting a One Health approach as a central aspect of their preparedness strategy. In this context, PREDICT brought together a multidisciplinary team of professionals in human, animal, and environmental health from the Interstate School of Veterinary Science and Medicine (EISMV), the Cheikh Anta Diop University of Dakar, and the Senegalese Institute of Agricultural Research/Laboratory for Animal Husbandry and Veterinary Research (ISRA/LNERV) to implement One Health surveillance activities at high-risk animal-human interfaces between January 2017 and September 2019. These activities were designed to strengthen capacity for detection of emerging viral threats and to assess risk of zoonotic diseases in communities with close and frequent animal contact.

Through analysis of project data and findings, the PREDICT team was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

LOCAL PARTNERS

- Cabinet du Premier Ministre, Sénégal
- Defense Threat Reduction Agency/Cooperative Biological Engagement Program
- Direction des Parcs Nationaux
- Institut Pasteur de Dakar, Dakar
- Ministère de l'Agriculture et de l'Equipment Rural
- Ministère de l'Elevage et des Productions animales
- Ministère de l'Environnement et du Développement Durable
- Ministère de la Santé et de l'Action Sociale
- Organisation des Nations Unies pour l'Alimentation et l'Agriculture (FAO), Dakar
- Organisation Mondiale de la Santé Sénégal (WHO), Dakar
- REDISSE (World Bank), Dakar
- UCAD/Hôspital Aristide le Dantec, Dakar
- US CDC, Dakar
- USAID EPT One Health Workforce
- Réserve de Bandia

Global Health Security Agenda

>70

DEVELOPED the One Health Workforce by training more than 70 people in Senegal.

>1.6K

OPERATIONALIZED One Health surveillance and sampled over 1.6K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Cheikh Anta Diop University of Dakar (UCAD)
- Senegalese Institute of Agricultural Research/Laboratory for Animal Husbandry and Veterinary Research (ISRA/LNERV)

23,511 TESTS

5 PREDICT

9 KNOWN

DETECTED 14 unique viruses in both animal and human populations.

MODOU MOUSTAPHA LO

ISRA PI, *Institut Sénégalais de Recherches Agricoles*

"PREDICT has been the most involved program in the implementation of the One Health approach in Senegal. The PREDICT/Senegal team, representing the three main Senegalese human, animal and environmental health structures in Senegal, has been involved in four out of the six priority zoonotic diseases in the country."

MAME CHEIKH SECK

Human Sampling Lead, *Université Cheikh Anta Diop de Dakar*

"The One Health approach became a reality in Senegal. Thanks to PREDICT, different health professionals (doctors, veterinarians and ecologists) united efforts to monitor, detect and respond to endemic threats in the wildlife-human interface."

AMADOU ALASSANE N'DIAYE

Sampling Lead, *Interstate School of Veterinary Science and Medicine (EISMV)*

"PREDICT has given me the opportunity to get deeply involved in research, community awareness and capacity building. PREDICT has a lasting impact on the consolidation of the One Health approach in Senegal. I have had an incredible work experience with PREDICT which will serve me well during my career."

ACHIEVEMENTS

- Trained the first multidisciplinary team (medical doctors, veterinarians, environmentalists, biologists, etc.) to safely conduct wildlife surveillance in Senegal
- Established an operational workforce to monitor priority viral pathogens that may emerge from wildlife
- Sampled 823 humans in clinics and in the community
- Sampled 874 wild animals (bats, rodents, and non-human primates)
- Tested 11,520 human samples at the UCAD laboratory
- Tested 11,991 wildlife samples at the ISRA laboratory
- Trained 26 National Parks, Water and Forest officers in wildlife monitoring and sampling following strict biosafety and biosecurity measures
- Trained 25 technicians and students on laboratory techniques to identify viruses with epidemic potential following strict biosafety and biosecurity measures
- Engaged 865 people (notables, village chiefs, religious guides, youth and women's associations, workers, and students) in community outreach activities to reduce risk of viral spillover including providing information on how to live safely with wildlife
- Identified Human coronavirus 229E in bat samples
- Organized a One Health simulation exercise on how to react to the potential discovery of a filovirus of pandemic potential in wildlife, bringing together 45 diverse stakeholders: administrators, technical ministries, locally elected representatives, universities, technical partners, and donor agencies

ONE HEALTH SURVEILLANCE

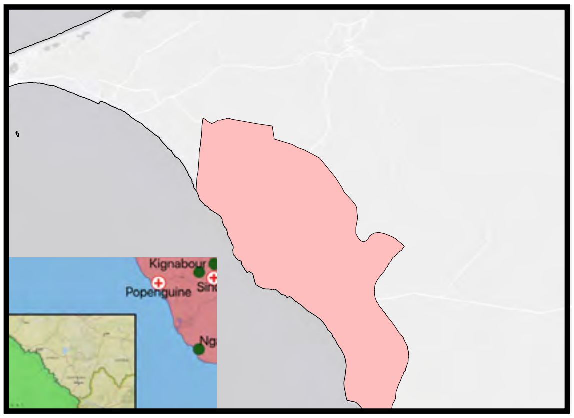


FIGURE 1. ***Concurrent One Health surveillance area in Sindia Prefecture, Senegal***

Together with in-country partners, the PREDICT project identified and characterized multiple animal-human high-risk interfaces including agricultural intensification, hunting, and ecotourism within the Sindia region. Given the proximity of these interfaces to heavily populated areas, including the capital city of Dakar, and the potential for pathogen spillover and spread, partners collectively agreed to focus concurrent One Health surveillance efforts in and around the regional health clinics in the Sindia region and the Bandia Reserve, a privately-owned wildlife reserve open to tourism, and surrounding villages (Figure 1).

Concurrent animal and human surveillance took place between May 2017 and October 2018. Wildlife surveillance targeted three taxa, namely bats, rodents and non-human primates (NHP). While NHP were only sampled at the Bandia Reserve, bat and rodent samples were collected at all sites within the Sindia Prefecture. Human surveillance occurred in regional health clinics (syndromic surveillance) as well as within the surrounding communities that sought care from these clinics (community-based surveillance). Syndromic surveillance took place within two regional health clinics and targeted malaria negative patients that presented with various symptoms including fever of unknown origin, encephalitis, and hemorrhagic fever. Patient sampling was safely conducted and included collecting biological specimens (oral, rectal, nasal swabs, and blood) and completing a standardized qualitative questionnaire on demographics, risk perceptions, behaviors, and interactions with animals.

HUMAN SURVEILLANCE

A total of 824 participants were enrolled. The majority were recruited in the villages (62%) with 219 (26%) sampled in the clinics. Study participants were mostly females (62%) with a median age of 26. Most people enrolled reported no education (30%) or indicated primary school as their highest educational level (36%). Specific demographics by site and season are presented in Table 1.

ANIMAL SURVEILLANCE

Throughout the PREDICT project, we safely and humanely sampled 874 wild animals, including 358 bats, 263 rodents, and 253 NHP. Genetic barcoding to confirm the host species identification was performed on all virus-positive individuals, and as possible on virus-negative animals. Insectivorous bats, such as Sundevall's roundleaf bat (*Hipposideros caffer*) and Noack's roundleaf bat (*Hipposideros ruber*) composed 57% of the bats sampled, while two fruit bat species, the straw-coloured fruit bat (*Eidolon helvum*) and the Egyptian fruit bat (*Rousettus aegyptiacus*), represented the remaining 43% sampled. House mice (*Mus musculus*) were the predominant rodent species sampled (68%), followed by black rats (*Rattus rattus*; 22%) and unidentified shrew species belonging to the Soricidae family (9%). Additionally, we sampled one African grass rat (*Arvicanthis niloticus*) and one giant pouched rat (*Cricetomys emini*). All NHP sampled were Green monkeys (*Chlorocebus sabaeus*).

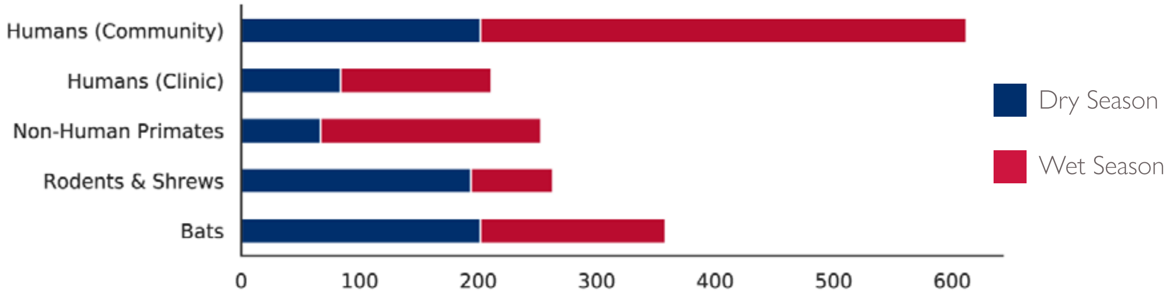
SURVEILLANCE FINDINGS

Searching for viruses in wildlife before they emerge in humans is core to the PREDICT project's One Health surveillance approach. We used broadly reactive consensus PCR supplemented with high throughput sequencing to screen for priority zoonotic viral diseases and emerging threats such as filoviruses (Ebola and Marburg), influenza viruses, coronaviruses, paramyxoviruses, and flaviviruses. These powerful tools produce specific, high-resolution data, allowing for detection of known and new potential pathogens.

In total, PREDICT laboratory partners at UCAD and ISRA/LNERV safely tested 23,511 specimens, of which 11,991 were collected from wildlife (5,859 bats, 4,361 rodents and 1,771 NHP) and 11,520 were collected from humans.

PARTICIPANT CHARACTERISTICS ACROSS ALL SURVEILLANCE SITES

SITE CHARACTERISTICS				PARTICIPANT DEMOGRAPHICS & SAMPLE CHARACTERISTICS					
SITE NAME	HUMAN DENSITY	SAMPLING TYPE	TOTAL	GENDER		SEASON		SPECIMENS COLLECTED	
				FEMALE (n)	MALE (n)	DRY (n)	WET (n)	NASAL SWABS (n)	ORAL SWABS (n)
SINDIA NORTH EAST	RURAL	COMMUNITY	196	116	80	61	135	196	196
SINDIA CENTRAL NORTH	RURAL	COMMUNITY	197	136	61	61	136	197	197
CLINIC - SINDIA WEST	PERI-URBAN	SYNDROMIC	7	3	4	3	4	7	7
CLINIC - SINDIA CENTRAL	PERI-URBAN	SYNDROMIC	211	113	98	78	133	211	211
SINDIA NORTH	RURAL	COMMUNITY	213	144	69	74	139	213	213



Numbers of individuals sampled by taxa group in Senegal

VIRUS DETECTION IN HUMANS

Of the 824 human participants, 7% (n=60) were positive for at least one virus. In total, five known viruses were confirmed, including strains of betacoronavirus 1 (OC43), influenza A, influenza B, human parainfluenzavirus 1 and Mumps virus (see Virus Table). Sixty-five percent (65%) (n=40) of participants that were found to be positive for a virus came from the syndromic surveillance at clinics. The majority of the positive cases were women, 67% (n=40) and were sampled during the wet season 92% (n=55). There were no positives from the clinic in the Sindia west region. Positive participants reporting previous contact with animals included 8% (n=5) having contact with rodents, 50% (n= 30) with poultry and other fowl and only 1% (n=1) with a NHP. Interestingly, none of the positive participants reported having contact with bats.

VIRUS DETECTION IN WILDLIFE

In wildlife, we found nine viruses in 33 animals, four are known viruses and five are previously unknown (see Virus Table). In rodents, a murine coronavirus and multiple new paramyxoviruses were confirmed. A new paramyxovirus and multiple strains of coronaviruses such as the alphacoronavirus coronavirus 229E were found in bats. No viruses were detected in NHP.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Clinic (Sindia Central)	2	0	2
	Bat coronavirus Hipposideros	Noack's Roundleaf Bat, Sundevall's Roundleaf Bat, Straw-Coloured Fruit Bat	Bandia Reserve, Sindia Northeast	15	1	14
	Coronavirus 229E (Bat strain)	Sundevall's Roundleaf Bat	Bandia Reserve	1	0	1
	Eidolon bat coronavirus	Straw-Coloured Fruit Bat	Ngaparou, Sindia Central	7	7	0
	Murine coronavirus	House Mouse	Sindia Central-north	1	0	1
Paramyxovirus	Human parainfluenzavirus 1	Human	Clinic (Sindia Central)	1	0	1
	Mumps virus	Human	Clinic (Sindia Central)	1	1	0
	PREDICT_PMV-174	Sundevall's Roundleaf Bat	Bandia Reserve	5	0	5
	PREDICT_PMV-167	Black Rat	Sindia Central	1	0	1
	PREDICT_PMV-169	Unidentified Crocidura Shrew	Sindia Central-north	1	0	1
	PREDICT_PMV-172	African Grass Rat	Sindia Central-north	1	0	1
	PREDICT_PMV-173	House Mouse	Bandia Reserve	1	0	1
Influenza virus	Influenza A	Human	Sindia Northeast, Sindia Central-north, Sindia North, Clinic (Sindia Central)	50	48	2
	Influenza B	Human	Clinic (Sindia Central)	6	6	0
Total				**93**	**63**	**30**

BEHAVIORAL RISK

Contact with animals either directly or indirectly was highly prevalent among people surveyed, with nearly all reporting interactions within the last year, both in the community and health clinic groups. Sixty percent (60%) of the interviewees reported to have pets in their dwellings, and some mentioned sharing water (10%) or food (3%) with these animals. Most participants (79%) did not report to know what the main source of diseases in their communities are, but suggested climate (5%), fatigue (5%), contact with sick people, and (3%) other factors. People's perception of disease transmission risk from live animals (wild and domestic) sold at their local markets was divided, with 56% of people reporting concern about sick animals. Interestingly, raw meat or meat originating from a sick animal is not regularly consumed, with only 4% of people reporting engaging in this risky behavior. When asked about contact with wildlife, 31% reported to have contact with rodents, 3% had contact with bats, and only 0.3% had contact with NHPs. However, reported contact varied from one surveillance site to another. For example, the highest contact with rodents was reported from Sindia north east, a semi urban area (20%), and the highest reported contact with bats was in Sindia north, a rural area (14%). The majority of people working in close animal-human contact interfaces were in the animal production (n=83) and crop (n=83) industries. Areas with crop production appear to be hotspots for potential animal to human disease transmission, with 72% of workers reporting that livestock and wildlife often frequent the farms, thus providing opportunities for contamination of products that are for human consumption. A small proportion of participants reported that they hunted animals (1% within the last year, and 8% over their lifetime). However, approximately a quarter of people whose livelihoods were linked to animals, such as crop farmers (25%) and animal production workers (19%), stated that bushmeat was available to consume on or near their work site.

Awareness of risk of disease transmission via bodily fluids through direct contact when slaughtering animals or through bites and scratches was uncommon (45% responded not being aware of a risk). However, although most people do not butcher or slaughter their own meat (76%), those that do (n=202) said they are likely to either visit a doctor (47%) if injured and/or self-clean a wound (42%), with only 8% taking no action. Most people interviewed seek health care from the local clinic (90%) or community health worker (12%). Of those that seek help from a traditional healer (6%, n=49), use was more common by women (76%).

KEY SURVEILLANCE FINDINGS THAT SUPPORT THE ONE HEALTH APPROACH

Two sites, Sindia central and Sindia northeast, were unique in several aspects, including being the sites with the most virus discoveries in both animals and people. They were also the only sites with positive samples from three taxa: bats, humans and rodents. Further studies are needed to understand whether the same viruses are shared across taxa. These two sites were the only sites with positive samples for three of our target virus families, influenza viruses, coronaviruses, and paramyxoviruses.

More samples collected during the wet season were found to be positive than those collected in the dry season. Influenza was mostly detected in the rainy season, whereas coronaviruses and paramyxoviruses were predominantly detected during the dry season.

STRENGTHENING CAPACITY

The PREDICT project convened various trainings in the field and laboratory, providing opportunities to strengthen skills across the full spectrum of surveillance, detection, and response. The PREDICT project deliberately designed a One Health approach that encouraged hands-on development of core skills lacking in the current animal and human health workforce in Senegal, as identified through the Joint External Evaluation. These trainings directly strengthened the capability of the current workforce to successfully and safely conduct core functions of their job on the frontlines of zoonotic disease surveillance and response in Senegal. A highlight of this effort was the first-ever One Health tabletop simulation exercise for "Detection and Response to a Filovirus Outbreak in Sindia". This event was co-organized with the Senegal Government's Emergency Operations Center (COUS) and involved 45 advisors from the Prime Minister's Office; the General Director of the Ministry of Health; representatives from the Ministries of Agriculture, Health and the Environment; CDC; WHO; and other Global Health Security Agenda partners.

PRACTICAL IMPLICATIONS

The PREDICT project strengthened Senegal's ability to detect and respond to zoonotic disease threats. EISMV, ISRA/LNERV, and UCAD have collaborated with key stakeholders and government partners to implement a One Health approach to disease surveillance.

Investigation into risky behaviors of individuals at the animal-human interface showed that contact with live animals is highly prevalent, but few people understand or acknowledge that this contact may lead to disease transmission. Community engagement activities targeting how to live safely with wildlife should be continued.

In terms of virus findings in Senegal, coronaviruses and paramyxoviruses were found in all sample types across both humans and animals. This is of potential interest for further virus investigations.

Linking the outcomes from the behavioral questionnaire with virus findings, there were no participants positive for a virus that self-reported having contact with bats, although the majority of PREDICT project sites included homes with bats roosting in them. It may be important to highlight this interface as a potential risk to the communities when continuing engagement activities.

For more information view the interactive report at **p2.predict.global**

PREDICT

SIERRA LEONE | EBOLA HOST PROJECT

ONE HEALTH IN ACTION (2016-2020)

Tracing the origins of Ebola

Nestled in the heart of coastal West Africa, Sierra Leone is a shining example of resilience and strength in the face of unprecedented health challenges. With nearly 5.7 million people living in a gradient of dense urban to very rural deep forest environments, Sierra Leone has long been recognized as a zoonotic virus emergence hot spot. During 2013-2016, what was initially thought to be a limited Ebola virus outbreak began along the Sierra Leone-Guinea-Liberia border. However, over the next three years the disease spread through nearly the entire three countries – leaving over 28,000 people infected and more than 11,000 dead.

This was the largest Ebola outbreak in history and further catalyzed efforts to identify the animal source, or reservoir, of Ebola to prevent future outbreaks. To meet this challenge, PREDICT designed and implemented the Ebola Host Project in Sierra Leone, along with neighboring Guinea and Liberia, to find the animal source of this and other devastating filoviruses, and to investigate human behaviors associated with viral spillover. A robust community engagement strategy was also undertaken, engaging over 400 stakeholders from the national, district, chiefdom, and local community levels. Additionally, our team worked closely with government officials in six districts to launch district-level One Health Platforms.

Through the University of Makeni and partners in the Ministries of Health and Sanitation and Agriculture and Forestry, PREDICT strengthened national capabilities for virus surveillance in wildlife, safely and humanely collecting samples from over 9,500 animals (bats, rodents, livestock, dogs, cats, and non-human primates) across >50 independent locations.

In 2018, as proof of concept for PREDICT's approach, we detected an entirely new species of ebolavirus (*Bombali ebolavirus*) in insect-eating bats; the first time an ebolavirus was discovered before causing human or animal illness or death. A few months later, our team together with colleagues at the United States Centers for Disease Control and Prevention (CDC) and Njala University, detected a deadly known virus (Marburg virus) in fruit-eating bats, the first time this virus had been detected in West Africa.

PREDICT's full impact in Sierra Leone will take years to fully appreciate. Through analysis of project data and findings, PREDICT was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats. Our staff continue to promote One Health as a key approach to obtaining health security through their roles as leaders in health, agriculture, and the environment.

LOCAL PARTNERS

- University of Makeni
- Ministry of Health and Sanitation
- Minsitry of Agriculture and Forestry

(Opposite) PHOTO: SIMON TOWNSLEY


PHOTO: SIMON TOWNSLEY

Global Health Security Agenda

250

DEVELOPED the One Health Workforce by training 250 people in Sierra Leone.

>9.5K

OPERATIONALIZED One Health surveillance and sampled over 9.5K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- University of Makeni
- UC Davis One Health Institute

29,160
TESTS

3 PREDICT **5** KNOWN

DETECTED 8 unique viruses in bats.

MOHAMED TURAY

Field Ecologist, *University of Makeni*

"It has been a pleasure working with PREDICT. I have learned a lot. I am not actually a scientist – I am an IT person, but I have build my system to love the science. I love my work that I am doing, it is nice and I am learning a lot. It gives me what it takes to reach to communities, offer what I have to offer. I love what I am doing."

FATMATA BAIROH

Field Ecologist, *University of Makeni*

"I have had an amazing work experience with PREDICT. The project was not all about us collecting animal samples but PREDICT gave back to me by building my capacity in lab and biosafety training. I can now identify the different species of bats through their external feature and their inhabitants. Ultimately with PREDICT, I became a better person when it came to team work."

ACHIEVEMENTS

- Discovered an entirely new species of ebolavirus (*Bombali ebolavirus*) in bats before any known human or animal illnesses or deaths (Goldstein, et al., 2018)
- Discovered a known and deadly human pathogen (Marburg virus) in bats for the first time in West Africa, more than 2,400 km from any known endemic area (Amman et al., 2020)
- Supported outbreak response efforts for a pig die-off and coordinated efforts between the Government of Sierra Leone's Central Veterinary Laboratory, USAID, and US Department of Agriculture to facilitate diagnostic testing for samples collected from dead animals; these efforts and confirmatory USDA testing led to the diagnosis of African Swine Fever, the first occurrence of this disease in Sierra Leone, which was swiftly reported to OIE in February 2020
- Trained >160 district-level government officials in One Health skills and supported the development of six district-level One Health Platforms, a collaboration between Ministry of Health and Sanitation, Ministry of Agriculture and Forestry, and the Environmental Protection Agency to sustain multi-sectoral coordination and bolster national health security
- Catalyzed efforts within the Ministry of Health and Sanitation's Health Education Division leading to the development of formalized risk messaging and communication campaigns for living safely with animals and preventing zoonotic disease transmission
- Served as a functional platform and role model of technical assistance that led to the the establishment of an emerging Zoonotic Disease Surveillance Task Force within the Ministry of Health and Sanitation's Emergency Operations Center, a monumental step towards proactive solutions to emerging pathogens at the national level

ONE HEALTH SURVEILLANCE

Finding the elusive wildlife hosts of ebolaviruses and other potential viral threats

PHOTO: SIMON TOWNSLEY

In collaboration with in-country partners, an extensive surveillance program was implemented in Sierra Leone to successfully identify the animal source and reservoir of Ebola virus and other closely related filoviruses (ebolaviruses and marburgvirus).

Across >50 locations in six districts, biological specimens were safely and humanely collected from >9,500 animals (bats, rodents, non-human primates, livestock, and domesticated animals such as dogs, cats, goats, and sheep) before being released.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Filovirus	Bombali virus (BOMV)	Angolan Free-Tailed Bat, Little Free-Tailed Bat	Gbindi, Kamabai, Robuya, Rosanda, Yelisanda	6	5	1
	Marburg virus (MARV)	Egyptian Fruit Bat	Kakoya, Koema, Peidu	5	2	3
Coronavirus	Bat coronavirus HKU9	Egyptian Fruit Bat	Kakoya, Peidu	5	5	0
	Coronavirus 229E (Bat strain)	Noack's Roundleaf Bat	Koema	1	1	0
Paramyxovirus	PREDICT_PMV-180	Angolan Fruit Bat	Koema	1	1	0
	PREDICT_PMV-183	Jones's Roundleaf Bat	Koema	1	1	0
Arenavirus	Lassa mammarenavirus	Natal Multimammate Mouse	Blama Puila, Kamaseh	5	4	1
Total				**24**	**19**	**5**

VIRUS DETECTION

Our team discovered an entirely new species of ebolavirus in Angolan free-tailed bats and Little free-tailed bats (*Mops condylurus,* n=1; *Chaerephon pumilus,* n=5), insect-eating bats in the Bombali district that were sampled in and around people's homes and the broader community. This discovery in 2018 marked the first time ever that an ebolavirus was found before causing any reported human or animal illnesses or deaths.

This new virus (*Bombali ebolavirus*) is now recognized as the sixth species of ebolavirus. Further characterization of the virus is ongoing to understand the zoonotic potential. Despite this finding and more than 40 years of research, data on the true reservoir host for the virus that caused the West African 2013-2016 outbreak (*Zaire ebolavirus*) remains elusive, though our team did detect *Zaire ebolavirus* in bats sampled in Liberia. The discovery of Bombali virus and detection of *Zaire ebolavirus* contributes to the growing body of evidence that bats are the likely hosts of this deadly group of viruses.

A few months after this discovery, PREDICT and partners with Njala University and the United States Centers for Disease Control and Prevention (CDC) detected the known and highly-lethal Marburg virus in five Egyptian fruit bats (*Rousettus aegyptiacus*) sampled at roosting sites in the Koinadugu, Kono, and Moyamba districts. This finding was significant, as the closest known endemic area for Marburg virus was more than 2,400 km east in Gabon. Following the discovery, PREDICT, CDC, and Njala worked closely with the Government of Sierra Leone to develop risk communication and messaging strategies for the public health sector (laboratories, hospitals, and emergency response teams) and the general population to raise awareness of this new health threat.

Samples from over 300 domestic animals (cats and dogs) were tested using consensus PCR (cPCR) to screen for filoviruses – no viruses were detected. Samples collected from wildlife were also tested using cPCR to screen for filoviruses, with a subset of samples screened for arenaviruses, coronaviruses, and paramyxoviruses. Virus findings were confirmed through genome sequencing and interpreted to better understand the relationship of the detected sequence to those from known animal and human pathogens. A total of seven viruses, four historically known and three PREDICT-discovered viruses, were detected in 24 animals.

FINDINGS IN BATS

The bat coronavirus HKU9 was found in five Egyptian Fruit Bats (*Rousettus aegyptiacus*). There is to date, also no evidence to suggest that this virus poses a threat to human health.

This virus is not related to the novel coronavirus strain (SARS-CoV-2) causing coronavirus disease (COVID-19) and leading to a public health emergency of international concern. Additionally, coronavirus 229E, known to cause respiratory illness in people, was found in one Noack's roundleaf bat (*Hipposideros ruber*). This particular virus strain infects bats but does not cause respiratory illness in people and is not considered a public health threat.

Additionally, two new unique paramyxoviruses were detected, one in a Jones's roundleaf bat (*Hipposideros jonesi*) and one in an Angolan fruit bat (*Lissonycteris angolensis*). There is no evidence that PREDICT_PMV-183 poses a threat to human health. Genetically, PREDICT_PMV-180 is related to henipaviruses, several of which are known to cause disease in animals and people (e.g. Nipah virus, Hendra virus) and genome sequencing is ongoing to further characterize this virus and the zoonotic risks it may pose, if any.

FINDINGS IN RODENTS

Lassa mammarenavirus was found in five Natal multimammate mice (*Mastomys natalensis*). This strain is known to cause Lassa hemorrhagic fever in humans. Testing for this virus was performed as part of the separately funded PREEMPT project, which is strengthening capacity for surveillance and detection of Lassa virus in Sierra Leone and working with an international team to develop Lassa virus risk models, prevention strategies and potential countermeasures to combat this public health threat.

IDENTIFYING BEHAVIORAL RISKS FOR VIRAL SPILLOVER & SPREAD

ANIMAL-HUMAN CONTACT QUESTIONNAIRE

Our team conducted interviews to learn more about human behaviors that may be associated with zoonotic disease transmission or viral spillover. A standardized questionnaire was used in interviews with 588 individuals (304 females and 284 males) at 20 sites (19 rural and one urban) across five districts, sites that our team also targeted for animal surveillance activities. A total of 82% of respondents in these communities reported raising animals with 77% reporting sharing water sources with animals for washing. The majority (64%) reported observing animal feces in or near food supplies, and 69% reported eating food after it was touched or damaged by animals. Twenty six percent (26%) reported being scratched or bitten by an animal in the past year and 23% reported trapping or hunting animals. These findings are particularly concerning as several of these communities are in areas known as hotspots for zoonotic diseases such as Lassa fever, a deadly virus transmitted to people by mice rodents in and around people's homes and food supplies, and where concentrated efforts have been focused for years on awareness, risk eduction, and behavior change.

> “Sometimes we find their droppings in our water, we throw the water away and other times we use the water to bathe, etc.”
>
> –Community member

> “They do fry them and sell them, sometimes they can cook them in the form of a soup, while sometimes it's being prepared as the way they prepared chicken and put it in the tray for sale. But what I normally see is the fried bats being sold.”
>
> –Community member

PHOTO: SIMON TOWNSLEY

IN-DEPTH BEHAVIORAL RISK INTERVIEWS

Additionally, our team conducted six focus group discussions and 94 in-depth ethnographic interviews in four communities within the districts of Bombali, Koinadugu, and the Western Area to explore risky human behaviors and people's interactions with wildlife. This formative ethnographic research led our team to identify communities at high-risk for contact with bats in the Bombali district, where we then focused investigations on two sites. In Bombali we conducted focus group discussions and in-depth interviews with 28 individuals with direct or indirect contact with bats, including bat hunters. It was clear that the interviewees were not aware of the potential health risks posed from bat-human contact. Interviewees shared that insectivorous bats were seen as pests, were commonly found roosting in roofs of homes, and their excreta (urine and feces) were contaminating food and water supplies.

Through interviews with the fruit bat hunters, we learned of more high-risk exposure to bats. While individuals were aware that bats were implicated in the Ebola epidemic, they did not have an adequate understanding of how zoonotic diseases are transmitted or of the health risks that bats pose. Members of both communities heard public health messages concerning bats and wildlife during the Ebola epidemic, but they seemed unsure of the messages' veracity or relevance. The infrequency of zoonotic spillover events presents a particular challenge for health communication around Ebola and wildlife. Furthermore, the knowledge-focused health interventions implemented during the Ebola outbreak seem to have fallen short of motivating long-term behavior change, as virtually all of the hunters had resumed bat hunting by the resolution of the outbreak.

COMMUNITY OUTREACH RISK COMMUNICATION

Following the discovery of the Bombali virus in bats, PREDICT worked closely with USAID, the Ministry of Health and Sanitation, and Ministry of Agriculture and Forestry to swiftly develop a communications plan and a behavior change intervention resource to help raise awareness among community members about ways to reduce disease risks associated with human-to-bat contact. To identify the most culturally appropriate, feasible, and effective intervention resource format, our team developed a framework for assessing potential materials, channels of communications, respective audiences, and core messaging. A moderated picture book format, delivered by a trusted community leader, was selected as the best tool to put into the hands of our local team and in-country stakeholders. A communications plan was developed with ministry partners to ensure a well-coordinated effort and timely discussions with community stakeholders, following the release of the new ebolavirus finding.

This new resource, *Living Safely with Bats*, leveraged the subject matter expertise of PREDICT's interdisciplinary One Health team who contributed technical content. All illustrations were developed by a team member trained in animal biology and visual arts to ensure accurate, consistent, and compelling visual representations throughout the book. Our Sierra Leone team took this resource into communities where the viruses were detected and met directly with each community and their respective district level government and chiefdom officials. This behavioral risk communication campaign utilizing *Living Safely with Bats* reached an estimated 1,000 individuals in 90% of communities engaged during the project. During the campaign, our team emphasized that while bats may harbor potentially infectious pathogens, they also play a very important role in the ecosystem by pollinating crops and reducing insects that can spread other diseases, such as mosquitoes. To reduce the risk of human exposure to potential pathogens, it was stressed that people should avoid direct contact with bats, their droppings or fluids, and food materials that have been fed upon by bats and other wildlife. In addition, our team shared messages for reducing risks of contact between bats and domestic animals and livestock, especially through indirect contact with bat excreta in animal feed, animal pens, and under orchards where animals graze and forage.

STRENGTHENING CAPACITY

PREDICT worked diligently to increase capacity for surveillance in animals and to strengthen testing capacity for filovirus detection in the country. Moreover, PREDICT recognized the importance of broader regional capacity with other nearby West Africa countries. In October 2017, PREDICT hosted a 10-day multi-national continuing education training, reaching more than 20 multi-disciplinary PREDICT staff from Senegal and Guinea at the University of Makeni. Participants were trained on safe and humane animal capture techniques, biological specimen collection and sampling, and safe transport of biological specimens from remote locations to virus detection laboratories with appropriate cold chain.

PREDICT also provided comprehensive training for district-level wildlife, medical, and government officers on community engagement, risk communication, and wildlife and livestock sampling across all 16 districts in Sierra Leone.

The training of One Health individuals that began with PREDICT has directly benefitted the government and citizens of Sierra Leone in combating SARS-CoV-2, the virus causing the COVID-19 pandemic. Currently, PREDICT-trained officers are working within four districts to support the tracking of COVID-19 cases in-country. These individuals have been instrumental in providing technical support to the WHO-Sierra Leone Surveillance Pillars at both the national and district levels, and in contact tracing and case investigation of COVID-19 positive individuals to mitigate virus spread.

PRACTICAL IMPLICATIONS

Further testing of newly discovered filoviruses, like Bombali virus, will greatly enhance our collective understanding of ‘pre-emergent’ virus diversity and the associated risks to human health. There remains much to understand about the specific factors leading to spillover of these types of viruses and the threat they may pose if people become infected. Working with partner institutions in the US government and other universities, PREDICT is working to determine the potential mechanisms used by Bombali virus could use to avoid immune responses leading to spread and possibly disease in bats and people. Understanding these “pathways of pathogenesis” are critical to develop effective countermeasures like vaccines or antiviral drugs to augment our preparedness in the face of potential future outbreaks. PREDICT proved that by putting extensive cross-sectoral and transdisciplinary One Health collaboration into practice at national, district, and community levels, Sierra Leone is now better positioned to improve national and global health security through zoonotic virus surveillance.

Communication about risks was an essential component of PREDICT´s approach to zoonotic disease surveillance, and it is encouraged that these efforts remain a central element for future vigilance efforts in Sierra Leone.

The *Living Safely with Bats* risk messaging book was highly valuable for community interventions to reduce and prevent exposures to potential pandemic viruses. Moreover, USAID’s Breakthrough Action project and the Sierra Leone Ministry of Health and Sanitation Health Education Division collaborated to adapt and broaden the message of the bat book by developing a *Living Safely with Animals*, risk messaging book. Similarly, the Ministry of Health and Sanitation developed and refined a communication campaign related to animal bites and living safely with animals. Initiatives focused on risk messaging will continue to strengthen prevention and preparedness for zoonotic diseases throughout the country.

The power of the PREDICT approach to disease surveillance and preparedness was recognized by the government of Sierra Leone and stimulated the creation of a Zoonotic Disease Surveillance Task Force within the Ministry of Health and Sanitation’s Emergency Operations Center. This was a monumental step towards proactive solutions to emerging pathogens in the country. With more attention, resources, and effort placed into One Health surveillance, the country and by extension the West Africa region will be further ahead of any potential zoonotic disease outbreaks.

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SPECIAL FEATURES

FILOVIRUS SURVEILLANCE IN SIERRA LEONE

Following the epidemic, PREDICT launched the Ebola Host Project (EHP) to conduct Ebola virus surveillance in livestock and wildlife in the three West African countries most affected by the outbreak. In 2018, EHP found a novel species of ebolavirus (*Bombali ebolavirus*) in Bombali, Sierra Leone in two species of insect-eating bats that were roosting in people's homes.

Learn more at **bit.ly/2SURLaC**

INSIGHTS ON THE GEOGRAPHIC DISTRIBUTION OF MOLOSSID BATS IN WEST AFRICA

In direct response to the detection of Bombali virus in Molossid bats and in recognition of the limited data available regarding bat distribution in the region, the Government of Guinea requested assistance identifying areas in the country and greater West Africa region at highest risk for viral spillover from bats.

Learn more at **bit.ly/3fH56Nw**

VIRUS X HUNTERS: OPERATIONALIZING ONE HEALTH IN EMERGENCY RESPONSE & SURVEILLANCE

In Sierra Leone, the Ebola Host Project (EHP), which is part of the larger PREDICT project, was implemented to identify the animals that may act as reservoirs or transmission hosts for Ebola and other filoviruses, to further understand the spread of the virus and recommend prevention measures as well as strengthen in-country capacity to prevent, detect and respond to emerging threats.

Learn more at **bit.ly/3bpb0zw**

For more information view the interactive report at **p2.predict.global**

PREDICT
TANZANIA

ONE HEALTH IN ACTION (2009-2020)

A One Health approach to strengthening national health security

Since 2009, UC Davis, the Sokoine University of Agriculture (SUA), and government partners have worked to advance One Health capacity and wildlife laboratory infrastructure in Tanzania. During PREDICT-1, our One Health team targeted high-risk wildlife-human interfaces, collected samples from wildlife, and tested them for viral threats. In the first five years, 63 viruses were detected (12 known viruses and 51 new viruses).

Building on these successes, our team partnered with the Ifakara Health Institute (IHI) to launch intensive One Health surveillance of both animal and human populations in Tanzania's Lake Zone; this area is considered a hotspot for viral spillover and spread due to land conversion, human population movement from neighboring Uganda, Rwanda, and Burundi, and intensive livestock development. Our team's work has provided proof-of-concept for applying the One Health approach in Tanzania, strengthened subnational and district-level One Health platforms by training animal and human health professionals on the frontlines of zoonotic disease transmission, shed light on the viruses and pathogens circulating in animal and human populations in at-risk communities, identified human behaviors and practices that increase risk for zoonotic disease transmission, and delivered critical data and insights for strengthening health security and refining national surveillance plans.

In addition, by putting stakeholder engagement and community outreach at the forefront of our approach, we worked directly with communities at all levels to identify practical and actionable disease prevention and control strategies.

LOCAL PARTNERS

- Ifakara Health Institute
- Sokoine University of Agriculture
- University of California, Davis
- Clinic (Kigoma Rural District)
- Clinic (Kyerwa District)
- Food and Agriculture Organization
- Ministry of Health, Community Development, Gender, Elderly & Children
- Ministry of Livestock, Agriculture & Fisheries
- National Institute of Medical Research
- Tanzania National Parks Authority
- Tanzania Veterinary Laboratory Agency
- Tanzania Wildlife Research Institute

>260

DEVELOPED the One Health Workforce by training more than 260 people in Tanzania.

>5.7K

OPERATIONALIZED One Health surveillance and sampled over 5.7K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Ifakara Health Institute
- Sokoine University of Agriculture

34,369
TESTS

60
PREDICT

17
KNOWN

DETECTED 77 unique viruses in both animal and human populations.

HAPPY RAPHAEL MKALI

Laboratory Lead, *Ifakara Health Institute*

“What I really enjoy about working with PREDICT is the beauty of interacting with teams of different disciplines – from veterinarians to social scientists to public health professionals, who all together have brought different experiences on how to approach zoonoses and other One Health issues around our communities.”

MWOKOZI MWANZALILA

Behavior Scientist & Community Engagement Liaison, *Sokoine University of Agriculture*

“PREDICT project has helped me to create more confidence when talking in front of people. In addition, I have gained new knowledge on zoonotic diseases.”

ACHIEVEMENTS

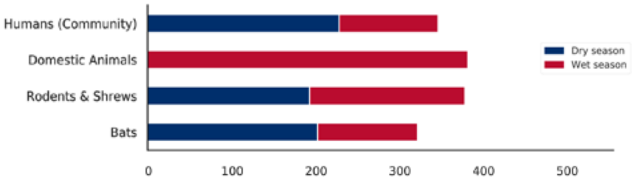
- Trained over 260 individuals in One Health skills
- Safely sampled >5,700 individuals (animals and people)
- Interviewed >1,600 people on behaviors and practices associated with viral transmission and spread
- Strengthened two research labs essential for supporting the national laboratory system
- Detected 77 viruses (60 new and 17 known) and enhanced national understanding of exposure to priority zoonotic diseases (Rift Valley fever and other viral hemorrhagic fevers)
- In partnership with the One Health Coordination Desk, empowered district-level One Health teams with the skills and knowledge to sustain zoonotic disease surveillance and strengthen multi-sectoral communications
- Contributed to COVID-19 response and SARS-CoV-2 testing capacity in Tanzania

ONE HEALTH SURVEILLANCE

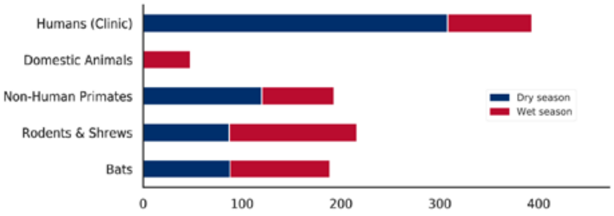
PREDICT's One Health surveillance was designed to train, equip, and enable the workforce to collect data and build the evidence base for priority zoonoses and emerging diseases in vulnerable and high-risk areas. PREDICT engaged local communities in high-risk locations and fostered improved recognition of zoonotic diseases and awareness of transmission pathways including prevention and control options. In addition, by sharing animal and human surveillance findings, PREDICT helped catalyze information and knowledge exchange between animal and human surveillance sectors.

In Tanzania, One Health surveillance sites were located in three distinct areas across the Lake Zone, an area considered a hotspot for viral spillover and spread due to land conversion, intensive livestock development, and human migration from neighboring Uganda, Rwanda, Democratic Republic of the Congo, and Burundi. To enable assessment of potential trends in viral spillover and spread across space and time, we collected samples from animals and people concurrently over a five-year period during both rainy and dry seasons.

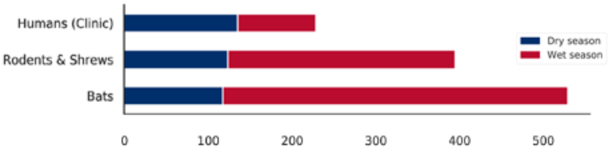
KIBONDO DISTRICT



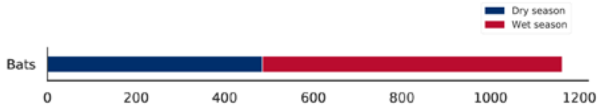
KIGOMA RURAL DISTRICT



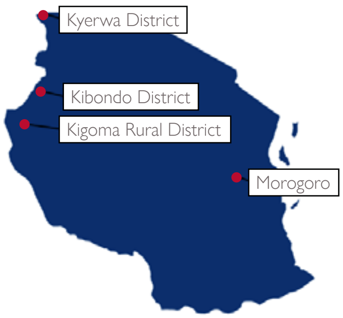
KYERWA DISTRICT



MOROGORO



Numbers of individuals sampled by taxa group



SURVEILLANCE SITES

KIBONDO DISTRICT = bats; cattle/buffalo; dogs; goats/sheep; humans; rodents/shrews; swine

KYERWA DISTRICT = bats; humans; rodents/shrews

KIGOMA RURAL DISTRICT = bats; cattle/buffalo; dogs; goats/sheep; humans; non-human primates; rodents/shrews

MOROGORO = bats

HUMAN SURVEILLANCE

Individuals were enrolled and tested at two local health centers (n=587, continuous enrollment) and from communities (n=294, episodic enrollment) where livestock and wildlife were sampled. At the health centers, we targeted patients presenting with fevers, while in community sites, we randomly selected individuals for participation. Children were only enrolled with parent or guardian consent at the health centers. Samples were collected from community members in Kibondo District, a rural area with game reserves and protected forests, where residents farm and raise animals, and where there are large settlements and established camps for refugees from neighboring countries. Sampling was also conducted within the Kigoma Rural District, which is home to both urban and rural communities. In the rural areas, residents largely farm and raise livestock. Febrile patients from both the urban and rural communities that enrolled at a clinic in the urban area were included in the syndromic surveillance.

Syndromic surveillance was also conducted at a clinic that serves rural communities within the Kyerwa District. These communities near the northwestern border of Tanzania engage in farming, the raising of animals, tin mining, logging, and charcoal production.

WILDLIFE & DOMESTIC ANIMAL SURVEILLANCE

Wildlife sampling locations were chosen near and adjacent to human surveillance sites. Rodents and bats were sampled at all three sites. In Kibondo, insectivorous bats were sampled from the roofs of domestic dwellings, government office buildings, and health care facilities; rodents were sampled at farms and near human dwellings. In the Kigoma Rural District, insectivorous bats were sampled from the roofs of dwellings, commercial offices, and a primary school. In addition, our team collected samples from baboons and other non-human primates in and around Gombe National Park, which is in the Kigoma Rural District where people were sampled. Livestock and canine samples were collected by the FAO and Government of Tanzania partners at sites within the Kibondo and Kigoma Rural Districts. In the Kyerwa District, frugivorous bats were sampled from an abandoned tin mine extraction cave that is adjacent to farms and orchards; rodents were sampled near dwelling and fields used for banana and maize cultivation. Finally, in Morogoro town, the home of Sokoine University of Agriculture, we conducted a targeted investigation into the seasonality of coronavirus shedding at an urban resident Straw-coloured fruit bat (*Eidolon helvum*) colony. At this site, our team collected bat feces from a tarp under the roosting colony each month over a 12-month period.

From 2014-2019, the PREDICT/Tanzania team safely collected and tested biological samples from a total of 881 people, 120 dogs, 52 cattle, 105 goats/sheep, 95 pigs, 911 bats, 193 non-human primates and 872 rodents, at the three concurrent surveillance sites in the Lake Zone. In Morogoro, 1,162 samples were non-invasively collected from frugivorous Straw-colored fruit bats. An additional 271 people completed structured behavioral risk questionnaires and 287 individuals were enrolled in focus group discussions and unstructured interviews using ethnographic techniques in Lake Zone communities within the Kibondo, Kyerwa, and Kigoma Rural Districts, where animals were sampled.

VIRUS DETECTION

Samples from wildlife, humans, and domestic animals were safely tested using consensus PCR (cPCR) to screen for priority zoonotic viral diseases and emerging threats such as filoviruses (Ebola and Marburg), influenza viruses, coronaviruses, paramyxoviruses, and flaviviruses. Virus findings were confirmed through genome sequencing and interpreted to better understand the relationship of the detected sequence to those from known animal and human pathogens.

Between 2014-2019, PREDICT Tanzania's One Health team established a safe and secure biobank of >23,000 samples from wildlife, domestic animals and people, including whole blood, oral and nasal swabs, urine, feces, and serum. Virus testing at PREDICT partner laboratories (SUA and IHI) focused on feces, nasal and oral swabs. Specimens from animals and people were analyzed through >41,800 cPCR tests across the five virus groups.

FINDINGS IN PEOPLE

Several known viruses that cause respiratory illnesses were detected in people in both health centers and the community: influenza A virus (n = 6), influenza B virus (n=1) and betacoronavirus 1 (OC43) (n=3). Influenza was more commonly detected in adult women in the rainy season. No viral infections were identified in children. In the Kibondo community, all influenza A infections detected were identified on two consecutive days in April 2018, consistent with an active respiratory infection moving through the community during the rainy season. One adult woman enrolled at the Kigoma District clinic who tested positive for malaria also tested positive for influenza A. influenza A was detected in oral swabs in three individuals and feces of four individuals, with one person having both specimens positive; influenza B was detected in an oral swab, and betacoronavirus 1 (OC43) was detected in oral and nasal swabs.

FINDINGS IN LIVESTOCK & DOMESTIC ANIMALS

Out of the 372 livestock and domestic animals tested, we detected six virus sequences for canine morbillivirus in domestic dogs (*Canis lupus familiaris*) at sampling sites in Kibondo. Canine distemper is a viral disease that affects many species of wild and domestic animals, and in dogs can cause symptoms ranging from mild to severe respiratory, gastrointestinal and neurological disease and death. This virus does not pose a threat to people but is relevant to wildlife conservation.

FINDINGS IN WILDLIFE

The PREDICT Tanzania team sampled and tested 911 bats, 872 rodents, and 193 non-human primates at concurrent surveillance sites. In rodents, we detected the coronavirus, PREDICT_CoV-64 in one pygmy mouse (*Mus minotoides*) and five unique paramyxoviruses in 10 rodents including multiple shrews (*Crocidura* sp., n=6), African grass rats (*Arvicanthis niloticus*, n=1), and the Natal multimammate mouse (*Mastomys natalensis*, n=3). Shrews were more likely to be positive for any virus among the rodents tested.

PREDICT_CoV-64 is a coronavirus belonging to the *Betacoronavirus* genus, found previously in rodents in Tanzania as part of the PREDICT project. The genus *Betacoronavirus* includes viruses that are of significance to public health such as SARS and MERS, however this virus is not considered to be closely related to either of these viruses. At this time there is no evidence to suggest this virus poses a threat to human health. There is no evidence at this time to suggest that any of the paramyxoviruses identified in rodents and shrews as part of the PREDICT project in Tanzania pose a threat to human health. No viruses were detected in any of the non-human primate samples tested.

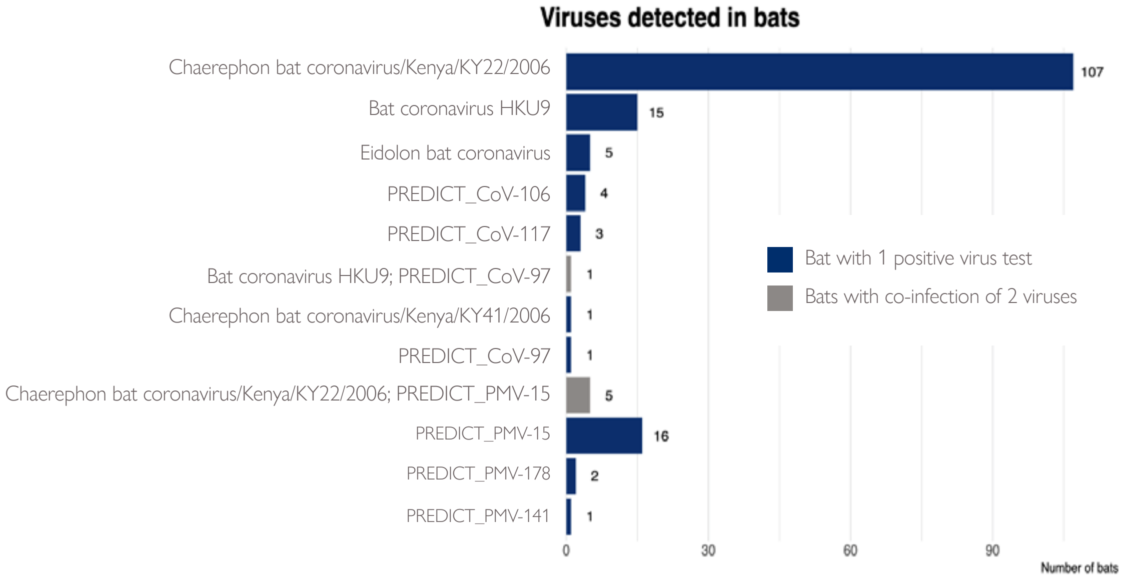
By far, the highest diversity of viruses identified and proportion of positive animals occurred in bats. Of 911 bats sampled and tested in the Lake Zone area, 161 bats were found to have positive virus findings. We detected seven different coronaviruses in 136 bats and three paramyxoviruses in 19 bats. One bat exhibited co-infection with two coronaviruses and five bats exhibited co-infection with both a coronavirus and paramyxovirus.

DETAILED VIRUS FINDINGS IN PEOPLE

	Influenza A (n=6)	Influenza B (n=1)	Betacoronavirus 1 (OC43) (n=3)	Negative (n=871)	Overall (n=881)
Season					
Dry	1 (16.7%)	0 (0%)	3 (100%)	613 (70.4%)	617 (70.0%)
Rainy	5 (83.3%)	1 (100%)	0 (0%)	258 (29.6%)	264 (30.0%)
Gender					
Female	4 (66.7%)	1 (100%)	1 (33.3%)	463 (53.2%)	469 (53.2%)
Male	2 (33.3%)	0 (0%)	2 (66.7%)	408 (46.8%)	412 (46.8%)
Age					
Adult (≥18)	6 (100%)	1 (100%)	3 (100%)	743 (85.3%)	753 (85.5%)
Child (<18)	0 (0%)	0 (0%)	0 (0%)	128 (14.7%)	128 (14.5%)
Site					
Kibondo	4 (66.7%)	0 (0%)	1 (33.3%)	289 (33.2%)	294 (33.4%)
Kyerwa	1 (16.7%)	1 (100%)	0 (0%)	192 (22.0%)	194 (22.0%)
Kigoma Rural	1 (16.7%)	0 (0%)	2 (66.7%)	390 (44.8%)	393 (44.6%)

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Kibondo District, Clinic (Kigoma Rural District)	3	0	3
	PREDICT_CoV-97	Egyptian Fruit Bat	Kyerwa District	2	0	2
	PREDICT_CoV-106	Angolan Free-Tailed Bat	Kibondo District	4	0	4
	PREDICT_CoV-117	Egyptian Fruit Bat	Kyerwa District	3	3	0
	Bat coronavirus HKU9	Angolan Free-Tailed Bat, Egyptian Fruit Bat	Kibondo District, Kyerwa District	16	12	4
	Chaerephon bat coronavirus/Kenya/KY22/2006	Angolan Free-Tailed Bat, Egyptian Fruit Bat, Little Free-Tailed Bat	Kibondo District, Kyerwa District, Kigoma Rural District	112	39	73
	Chaerephon bat coronavirus/Kenya/KY41/2006	Angolan Free-Tailed Bat	Kibondo District	1	0	1
	Eidolon bat coronavirus	Egyptian Fruit Bat, Straw-Coloured Fruit Bat	Kyerwa District, Morogoro	130	73	57
	PREDICT_CoV-64	Pygmy Mouse	Kibondo District	1	1	0
Paramyxovirus	PREDICT_PMV-15	Angolan Free-Tailed Bat	Kibondo District, Kigoma Rural District	21	10	11
	PREDICT_PMV-141	Egyptian Fruit Bat	Kyerwa District	1	0	1
	PREDICT_PMV-178	Egyptian Fruit Bat	Kyerwa District	2	2	0
	PREDICT_PMV-143	African Grass Rat	Kyerwa District	1	1	0
	PREDICT_PMV-144	African Giant Shrew	Kyerwa District	5	4	0
	PREDICT_PMV-146	Montane White-Toothed Shrew	Kyerwa District	1	0	1
	PREDICT_PMV-150	Natal Multimammate Mouse	Kibondo District, Kigoma Rural District	2	1	1
	Rodent paramyxovirus MpR12	Natal Multimammate Mouse	Kyerwa District	1	1	0
	Canine distemper virus	Domestic Dog	Kibondo District	6	6	0
Influenza virus	Influenza A	Human	Kibondo District, Clinic (Kyerwa District), Clinic (Kigoma Rural District)	6	5	1
	Influenza B	Human	Clinic (Kyerwa District)	1	1	0
Total				**319**	**159**	**160**



Chaerephon bat coronavirus/Kenya/KY22/2006 was the most common virus detected in bats sampled in the Lake Zone and we confirmed sequences of this virus in samples from 112 bats. This is a strain of the known alphacoronavirus Chaerephon bat coronavirus/Kenya/KY22/2006 (Genbank Accession no. HQ728486) that has previously been found in bats. There is no evidence at this time to suggest this virus poses a threat to human health. This virus was found in a several bat species including insectivorous Angolan free-tailed bats (*Mops condylurus*, n = 94) and little free-tailed bats (*Chaerephon pumilus*, n = 17) as well one Egyptian fruit bat (*Rousettus aegyptiacus*). This virus was detected in 111 bats sampled during both the rainy and dry seasons in the Kibondo and Kigoma Rural Districts. The detection of this virus in a single Egyptian fruit bat sampled in abandoned mine shaft in the Kyerwa District is notable as the species and sampling environment are divergent from the rural and peri-domestic sites in the Kibondo and Kigoma Rural Districts. Rectal swabs were most likely to yield a positive virus finding for this virus, with 90 bats positive only on rectal swab, 17 bats positive on oral swab, and five bats with positive oral and rectal swabs.

In addition to Chaerephon bat coronavirus/Kenya/KY22/2006, PREDICT Tanzania identified two additional alphacoronaviruses, four betacoronaviruses, and three paramyxoviruses. There is no evidence at this time to suggest any of these viruses pose a threat to human health, however given the recent emergence of novel betacoronavirus SARS-CoV-2, additional investigation into the ecology, evolution and global distribution of betacoronaviruses in wildlife is warranted. Further analysis of the betacoronaviruses PREDICT_CoV-106 and PREDICT_CoV-117 identified in Tanzania is ongoing at partner laboratories at UC Davis and Columbia University.

EPIDEMIOLOGICAL & BEHAVIORAL RISK

In addition to biological specimen collection, our PREDICT teams worked with local communities to learn about and better understand social and behavioral factors that might be associated with risks of zoonotic disease transmission, focusing on the “how” and “why” of risk. Using data-driven ethnographic and epidemiological methods, our team also worked to identify potential strategies that might be effective in preventing or mitigating zoonotic viral spillover and spread. Our team conducted qualitative (field observations, ethnographic interviews and focus group discussions) and quantitative (structured survey) behavioral risk investigations at all One Health surveillance sites.

HEALTH CENTER SURVEILLANCE

Of the 587 syndromic individuals enrolled and tested from the clinics in Kigoma Rural District (n=393) and Kyerwa District (n=194), 565 had fevers (≥38°C) on admission, with an average fever of 38.6°C of three days duration. The most common presenting symptoms at enrollment included: 1) fever, 2) headache, 3) malaise, 4) cough, and 5) joint or abdominal pain.

528 syndromic enrolled and tested humans were also tested for malaria using a malaria rapid diagnostic test; 38.1% of those tested for malaria were positive. All these humans were included for testing using our cPCR virus testing protocols.

ENROLLED & TESTED INDIVIDUALS BY AGE & GENDER

SITE ENROLLED	Kibondo District Community (n=294)	Clinic (Kyerwa District) (n=194)	Clinic (Kigoma Rural District) (n=393)	Total (n=881)
FEMALE	61 (20.7%)	112 (57.7%)	296 (75.3%)	469 (53.2%)
MALE	233 (79.3%)	82 (42.3%)	97 (24.7%)	412 (46.8%)
ADULT	294 (100%)	147 (75.8%)	312 (79.4%)	753 (85.5%)
CHILD (<18)	0 (0%)	47 (24.2%)	81 (20.6%)	128 (14.5%)
MEDIAN [MIN, MAX]	39 [18,76]	24 [2,82]	30 [2,88]	32 [2,88]

SELF-REPORTED CAUSE OF ILLNESS

For those >12 years old reporting an illness in the past year, a variety of responses were received. Across all education levels (none, primary, or secondary and above), the top five beliefs regarding cause of illness were: 1) don't know, 2) weather, 3) bad food or water, 4) infectious agents, and 5) insects.

LIVELIHOODS OF SURVEYED INDIVIDUALS

Across all sites, the primary livelihood of both women and men surveyed was farming and crop production. Men were significantly more likely to be engaged in livestock production, meat processing businesses, and forestry or extraction activities. Women on the other hand, were more likely to be engaged in the wildlife trade, in trade, marketing, or restaurant businesses, or to be employed as a health care worker. Additional livelihoods reported by respondents included: homemaker, sales, construction, migrant laborer, and protected area worker. Several individuals reported being students or unemployed.

SELF-REPORTED ANIMAL-HUMAN CONTACT

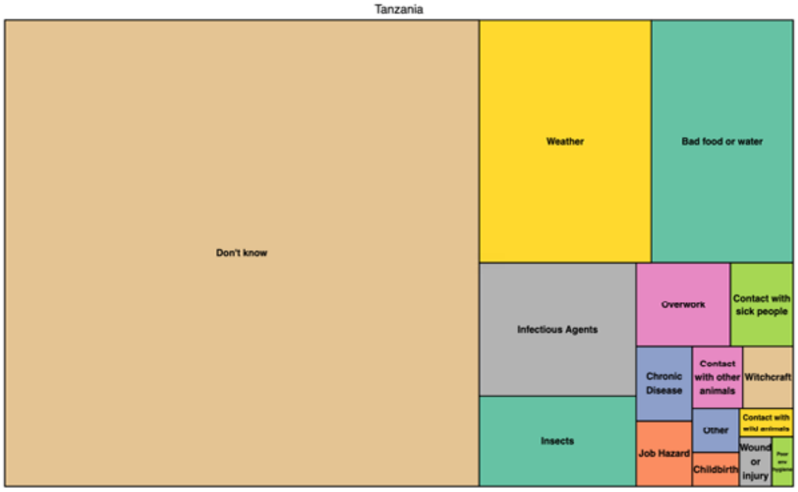
Respondents were asked about the type of contact they had experienced with animals within the last 12 months. Across all sites, rodents were the most common type of wildlife encountered by both men and women. Both genders commonly had contact with poultry livestock, with men more likely to have contact with all classes of animals: ruminants, bats, wild ungulates (antelope, buffalo, etc.) and non-human primates. Respondents self-reported their behaviors following an animal scratching or biting them, or after experiencing a cut or injury when butchering or slaughtering an animal; the majority of respondents (both men and women) answered that they took no preventive action.

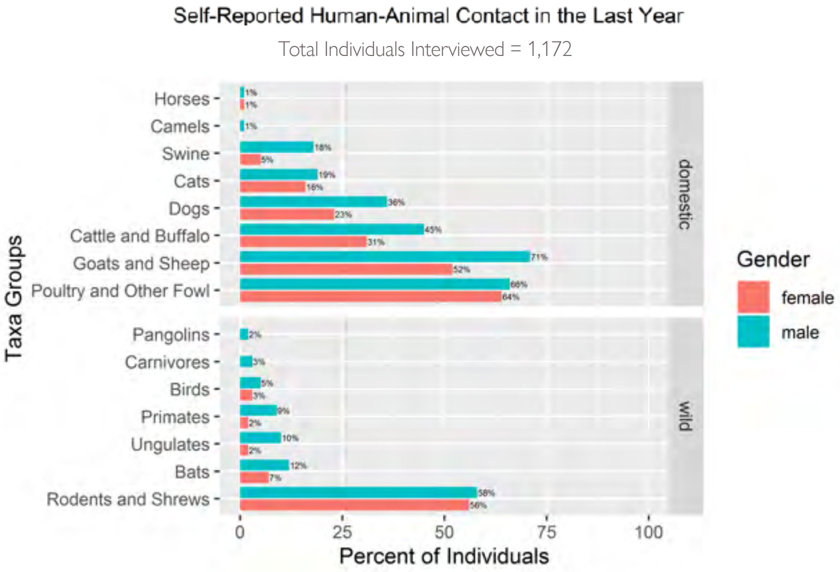
ANIMAL-HUMAN CONTACT AT HIGH-RISK INTERFACES

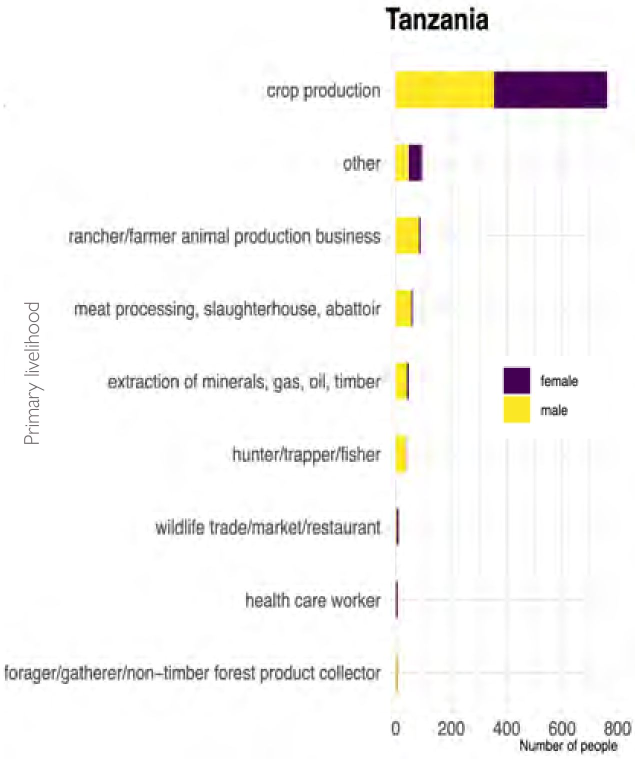
During ethnographic interviews and focus groups, many respondents spoke about the increasing scarcity of wild meat and attributed this to increased human population density. Other respondents also shared insights and stories about wildlife raiding their crops. Destruction of crops by baboons and other non-human primates, rodents, and both wild and domestic ungulates is such a severe problem that some farmers spend anywhere from several weeks to several months living in temporary shelters in their fields to scare animals away. This behavior comes with its own set of health risks, as farms lack shelter and are exposed and are far away from sources of clean drinking water and healthcare services.

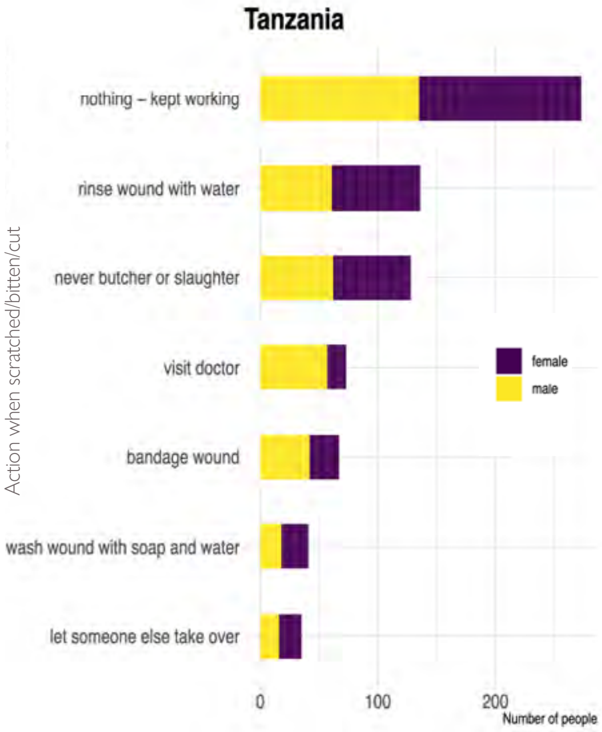
FOOD SAFETY, HUNTING & WILD MEAT CONSUMPTION

Across sites, interviewees described a range of conditions under which people eat the meat of animals known to have died of disease. While many communities have at least limited access to the services of livestock extension officers and community health workers who inspect meat after slaughter and prior to sale, respondents report that many animals are eaten without inspection, or possibly even following condemnation. Another pervasive theme is the claim that refugee populations drive hunting and bushmeat consumption, particularly of non-human primates.




Self-Reported Human-Animal Contact in the Last Year
Total Individuals Interviewed = 1,172
Taxa Groups
Horses
Camels
Swine
Cats
Dogs
Cattle and Buffalo
Goats and Sheep
Poultry and Other Fowl
Pangolins
Carnivores
Birds
Primates
Ungulates
Bats
Rodents and Shrews
1%
1%
1%
18%
5%
19%
16%
36%
23%
45%
31%
71%
52%
66%
64%
2%
3%
5%
3%
9%
2%
10%
2%
12%
7%
58%
56%
domestic
wild
0
25
50
75
100
Percent of Individuals
Gender
female
male


Tanzania
Primary livelihood
crop production
other
rancher/farmer animal production business
meat processing, slaughterhouse, abattoir
extraction of minerals, gas, oil, timber
hunter/trapper/fisher
wildlife trade/market/restaurant
health care worker
forager/gatherer/non–timber forest product collector
female
male
0
200
400
600
800
Number of people


Tanzania
Action when scratched/bitten/cut
nothing – kept working
rinse wound with water
never butcher or slaughter
visit doctor
bandage wound
wash wound with soap and water
let someone else take over
female
male
0
100
200
Number of people

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

PREDICT was committed to community engagement and worked with local villages and partners to raise awareness of zoonotic disease threats and strengthen capacity for prevention, surveillance, and detection. Several of the bat species sampled by our team are known hosts for deadly or potentially dangerous zoonotic diseases; namely Egyptian fruit bats, which are reservoirs for Marburg virus, and Mops species bats, which have been documented as wildlife hosts for a newly detected ebolavirus (Bombali virus) in Guinea, Sierra Leone, and neighboring Kenya. We thus identified the need to provide risk reduction strategies targeting bats. Our team developed a communications plan and a behavior change resource to raise awareness among community members about ways to reduce disease risks associated with human-to-bat contact, while highlighting the need for bat conservation given their roles in critical ecosystem services.

A moderated picture book format, delivered by a trusted community leader, was selected as the best tool to put into the hands of our local team and in-country stakeholders. This new resource, *Living Safely with Bats* was piloted in Sierra Leone and by our team in Tanzania to garner input on ways to improve both the content and the process of delivery.

In August of 2019, outreach and risk reduction campaigns were conducted with all communities and subnational health professionals. At these events, our team shared project findings, the *Living Safely with Bats* resource, and additional educational materials designed to raise awareness of zoonotic transmission risks from other animals. Broader findings and recommendations from the project were also shared with regional and national stakeholders, including the One Health Coordination Desk, which was established under the Prime Minister's office to strengthen multi-sectoral coordination and health security.

CAPACITY STRENGTHENING

From 2009-2020, PREDICT provided training to over 260 individuals across both animal and human health sectors and reached district-level professionals (veterinary officers, clinicians, game officers, etc.) on the front lines of the national disease surveillance system.

PREDICT trainings directly strengthened workforce capabilities to successfully and safely conduct core job functions and provided opportunities for students, interns, health professionals, and staff to strengthen skills in zoonotic disease surveillance and detection through hands-on learning in real world field-based settings.

PREDICT's labs, based at SUA and IHI, increased capacity for virus detection of both known and newly emerging zoonotic disease threats, and together have tested samples from over 5,200 animals and people. Both labs serve as key training centers for students and professionals, including government staff from the national lab system and standby to support the national lab system as referral nodes and technical assistance centers.

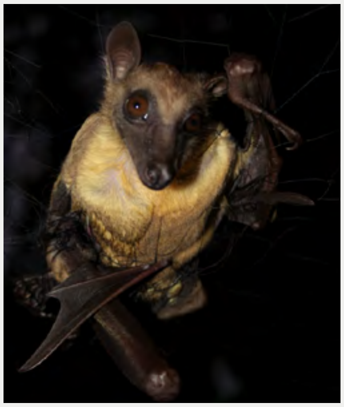
Read more about how our team supports women and science and contributes to COVID-19 response efforts at **p2.predict.global/strengthening-health-security**

PRACTICAL IMPLICATIONS

- Our team collected samples from several rodent and bat species known as hosts for deadly or potentially dangerous zoonotic diseases in an area bordering an active Ebola virus outbreak
- Bats and other wildlife are very common in villages and dwellings in this area, and we identified multiple high-risk pathways for viral transmission from wildlife to people, along with insights into peak season for viral spillover from bats, critical information for targeting cost-effective disease surveillance and timing of intervention strategies
- Despite these risks and nationally organized Ebola virus sensitization and outreach campaigns, awareness of zoonotic disease transmission risks remains low and surveillance for emerging viral threats in these at-risk populations is virtually non-existent
- We recommend continued investments in surveillance of these populations, especially wildlife using the One Health approach, along with focused investments in capacity strengthening for the subnational workforce and nascent One Health Platform to empower prevention, surveillance and detection in vulnerable transboundary areas
- Finally, at request of our community stakeholders, those living day to day in these high-risk animal-human interfaces, we recommend investments in interventions targeting risk reduction coupled with evaluations of education, social marketing, and behavior change communications for general health and disease prevention; interventions that might be taken to scale across the region via local schools and community organizations

SPECIAL FEATURE

PEAK SEASON FOR VIRAL SPILLOVER



In Tanzania, we worked together with scientists from UC Davis and Ghana to assess the seasonality of coronavirus shedding by the straw-coloured fruit bat (*Eidolon helvum*). Our teams passively collected ~100 fecal samples on a monthly basis during an entire year at two urban colonies: one in Accra, Ghana and one in Morogoro, Tanzania. Our data provides evidence that there is an association between coronavirus shedding in urban *E. helvum* roosts and the reproductive cycle of these bats, as coronaviruses are shed at a rate four times higher when pups are weaned compared to the rest of the year; an important finding for timely and effective disease control and prevention strategies.

Learn more at **bit.ly/2O2creo**

For more information view the interactive report at **p2.predict.global**

PREDICT
UGANDA

ONE HEALTH IN ACTION (2009-2020)

In Uganda, there are many opportunities for pathogens to emerge from wildlife that could pose a threat to human health. Uganda has a long-standing and well-established network of national parks, which provide protection for wildlife but are surrounded by dense human communities and intensive animal agriculture. Wildlife leave protected areas to forage in crops, and people live and enter parks for their livelihoods; this movement results in significant interaction and conflict among wildlife, livestock, and people. Uganda also supports a thriving wildlife ecotourism industry that brings both local and foreign people into daily contact with wildlife, including bats and primates, which present a zoonotic disease risk.

Since 2009, our team has been conducting field surveillance, building the One Health workforce, and strengthening systems for priority zoonotic viral diseases and other emerging threats in Uganda. During PREDICT, we put One Health into action through zoonotic disease surveillance in wildlife and people, including behavioral risk investigations at high-risk animal-human transmission interfaces. This work has directly supported the Uganda national surveillance and laboratory systems, contributed to the development and implementation of the country's GHSA roadmap, and provided opportunities to strengthen mechanisms for responding to priority zoonotic diseases, such as avian influenza and hemorrhagic fevers (e.g. Ebola, Rift Valley fever, and Marburg).

Working together, our team has supported the growth of Uganda's One Health workforce from the national to district level through field- and lab-based training, and engagement of national, university, and non-governmental organization (NGO) staff and students in our surveillance and disease detection activities. PREDICT Uganda trained wildlife veterinarians in the principles and practice of safe and effective wildlife surveillance, improved facilities, and acquired equipment for the safe, secure transport and storage of biological samples.

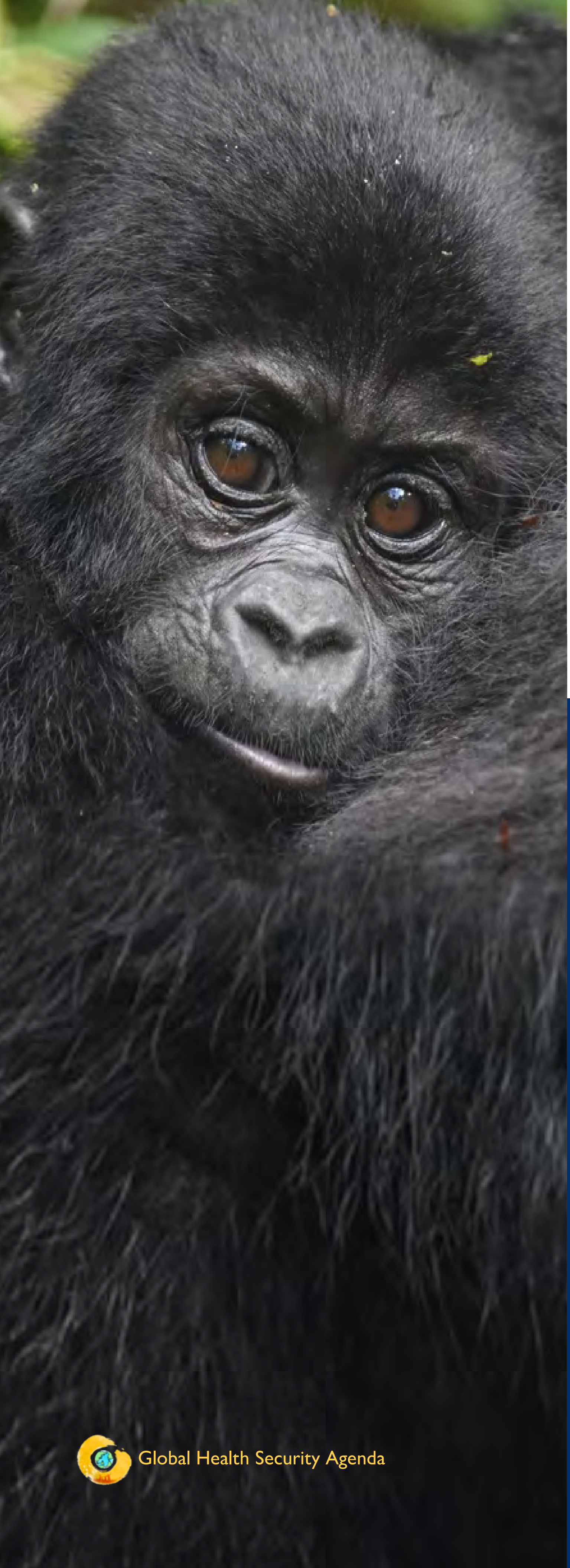
To better understand the threat posed to human health by viral pathogens circulating in wildlife, PREDICT Uganda collected samples from wildlife in areas where wild animals had significant interactions with domestic animals and people. From 2009-2019, PREDICT Uganda sampled over 2,900 wild animals (>1,243 non-human primates, >900 bats, >760 rodents, and 60 other wildlife taxa); 80 camels; and over 400 febrile human patients. Sample processing and PCR testing was performed at Makerere University Walter Reed Project Laboratory and Uganda Virus Research Institute, with confirmatory sequencing performed at the University of California, Davis, and Columbia University. Animal and human samples were tested for up to 16 virus families/genera. In total, 59 viruses were detected, of which 20 were known and 39 were newly described viruses. Novel viruses belong to the adenovirus, coronavirus, herpesvirus, paramyxovirus, and rhabdovirus groups.

PREDICT Uganda also participated in several One Health disease investigations, including outbreaks of Ebolavirus (May 2011 and July 2012) and yellow fever (November 2010). The team contributed to behavioral risk and wildlife surveillance efforts to better understand the role that wildlife may have played in the outbreaks. In addition, these efforts enabled PREDICT staff to identify and relay the most effective strategies for prevention, preparedness, and response to communities and health professionals in order to protect people and wildlife from disease threats.

LOCAL PARTNERS

- Uganda Wildlife Authority
- Uganda Virus Research Institute
- Bwindi Community Hospital (Buhoma, Kanungu District)
- Makerere University Walter Reed Project
- Makerere University College of Veterinary Medicine
- Ministry of Agriculture, Animal Industry and Fisheries

**Photos in this report credited to Gorilla Doctors unless stated otherwise*



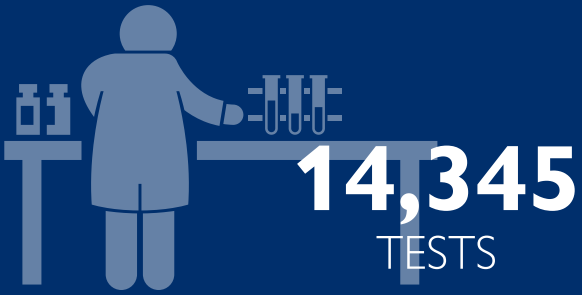
Global Health Security Agenda

DEVELOPED the One Health Workforce by training more than 70 people in Uganda.

OPERATIONALIZED One Health surveillance and sampled over 3.4K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Uganda Virus Research Institute
- Makerere University Walter Reed Project



DETECTED 59 unique viruses in both animal and human populations.

BENARD JASPER SSEBIDE

Country Coordinator, *Gorilla Doctors*

"Bwindi is one of those places where the potential for viral pathogens to emerge from forest wildlife and spillover into both local and transiting populations is very high. Surveillance to date has shown this to be true, and with our PREDICT project, we now better understand how that spillover occurs and therefore how best to prevent it."

RICKY OKWIR OKELLO

Field Veterinarian, *Gorilla Doctors*

"PREDICT surveillance has shown that timely and accurate detection and reporting of infectious disease outbreaks help guide interventions to control epidemics. Now that Uganda has veterinarians with the capacity to safely capture and sample wildlife, and the training of laboratory staff in protocols for virus screening, the country has moved a major step ahead towards preventing, detecting, and responding to various health security risks and public health emergencies of international concern."

ACHIEVEMENTS

- Supported the Uganda National Task Force for Epidemic Preparedness and Response in its investigations of several disease outbreaks to better understand the role that wildlife may play in these events, and to learn lessons for the future
- Strengthened the National Task Force by encouraging a One Health approach to disease outbreak investigation, control, and prevention, resulting in incorporation of wildlife investigations into disease outbreak response planning
- Discovered a new coronavirus in a bat that is the most closely related to MERS-CoV, which sheds light on bats as the potential reservoir for emerging infectious diseases like MERS, SARS and the current COVID-19 pandemic
- Identified 39 novel viruses in non-human primates, bats, and rodents, including several new adenoviruses, herpesviruses, paramyxoviruses, rhabdoviruses, and coronaviruses, as well as a known human coronavirus in a bat
- Developed a communication channel for sharing of surveillance test results with appropriate Government of Uganda ministries and departments

ONE HEALTH SURVEILLANCE

The overarching aim of PREDICT's One Health surveillance approach was to further build the evidence base for emerging infectious diseases and priority zoonoses at the wildlife-human interface. In Uganda, PREDICT focused its efforts in local communities living in close proximity to protected areas, working to better understand transmission pathways for viral spillover that could inform human disease prevention and control options.

Our surveillance work focused on the Southwestern districts of Kisoro, Rubanda, Kabale, Kanungu, and Rukungiri at sites where people and wildlife come into daily close contact through subsistence farming, wildlife tourism, and legal and illegal access to a highly biodiverse protected area, Bwindi Mgahinga Conservation Area. Bwindi and Mgahinga were fairly recently annexed by the Government of Uganda (in 1991) to serve as protected areas and the Batwa people who had previously lived and subsistence hunted in Bwindi were moved to communities surrounding the park. This part of Central-East Africa is among the most densely populated on the continent and is unique, as thousands of people come from around the world every year for the opportunity to view human-habituated mountain gorillas.

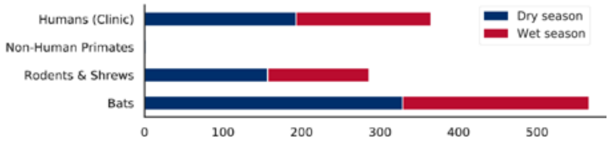
WILDLIFE & DOMESTIC ANIMAL SURVEILLANCE

To understand what viruses are circulating in wildlife that have the potential to infect humans, surveillance efforts focused on bats and rodents living in human dwellings and accessing agricultural grounds and crops in communities adjacent to Bwindi (primarily in Buhoma and Kisoro). From 2014-2019, a total of 572 wild bats (insectivorous and frugivorous) were safely and humanely captured in mist nets set up in neighborhoods, near roost sites, and across roads that people used to travel to, from, and through Bwindi. Likewise, 286 wild rodents were safely and humanely live-trapped in and around people's houses and croplands. In addition to intensive surveillance in the Bwindi area, there were specific opportunities to collect samples from wildlife utilizing highly urbanized environments (vervet monkeys in Entebbe), and from camels being utilized as pack animals, and thereby coming into very close contact with handlers and traders, in the Karamoja region (Moroto and Amudat Districts) of northeastern Uganda.

HUMAN SURVEILLANCE

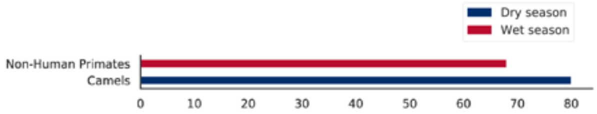
People living in communities adjacent to Bwindi Impenetrable National Park who presented to Bwindi Community Hospital with fevers of unknown origin were included in this concurrent One Health surveillance effort. Bwindi Community Hospital is located in the town of Buhoma, at one of the main gateways to Bwindi, and as one of the best health centers in the area, is accessed by people coming from multiple villages and towns surrounding the park. Bwindi Community Hospital also serves park personnel and their families, as well as the Batwa community. Patients who gave their consent for enrollment in the surveillance program provided biological samples (blood, mucosal swabs) and answered questionnaires about their demographics, livelihoods, and contact with animals (domestic and wild). Children (under the age of 18) were only enrolled with parent or guardian consent. From January 2017 to July 2018, 413 people were sampled and surveyed, including 107 adult men, 170 adult women, and 136 children.

GREATER BWINDI AREA



Numbers of individuals sampled by taxa

ENTEBBE & KARAMOJA



Numbers of individuals sampled by taxa

VIRUS DETECTION

Samples from wildlife, humans, and camels were safely tested using consensus PCR (cPCR) to screen for priority zoonotic viral diseases and emerging threats such as for filoviruses (Ebola, Marburg), coronaviruses (SARS, MERS), paramyxoviruses, flaviviruses, and influenza viruses. Virus findings were confirmed through genome sequencing and interpreted to better understand the relationship of the detected sequence to those from known animal and human pathogens. All tests were conducted at the Uganda Virus Research Institute (Entebbe, Uganda). Sequencing was conducted at the University of California, Davis and Columbia University. Test results were shared with the Government of Uganda through the appropriate ministries and departments and can be viewed at **www.data.predict.global**

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	TOTAL POSITIVE INDIVIDUALS
Coronavirus	Coronavirus 229E (Bat strain)	Noack’s Roundleaf Bat, Geoffroy’s Horseshoe Bat, Unidentified Hipposideros Bat	Rushaga, Kisoro	3
	Kenya bat coronavirus/ BtKY33/2006	Welwitsch’s Myotis	Kisoro	1
	Kenya bat coronavirus/ BtKY66/65/63/60	Welwitsch’s Myotis	Kisoro	1
	MERS-like coronavirus	Dusky Pipistrelle	Nkuringo	1
	PREDICT_CoV-43	Geoffroy’s Horseshoe Bat	Rushaga	7
	PREDICT_CoV-65	Geoffroy’s Horseshoe Bat	Rushaga	2
	PREDICT_CoV-70	Geoffroy’s Horseshoe Bat	Rushaga	1
	PREDICT_CoV-46	African Giant Shrew	Kisoro	1
	PREDICT_CoV-55	Unidentified Multimammate Mouse, Black Rat	Nkuringo, Nombe	2
	PREDICT_CoV-71	Toad Mouse	Nombe	1
Paramyxovirus	PREDICT_PMV-71	Geoffroy’s Horseshoe Bat	Rushaga	1
	PREDICT_PMV-68	Unidentified Crocidura Shrew, Stella Wood Mouse, Unidentified Swamp Rat	BINP	3
	PREDICT_PMV-69	Jackson’s Soft-Furred Mouse	BINP	1
	PREDICT_PMV-70	African Giant Shrew	Nombe	1
	PREDICT_PMV-76	African Giant Shrew	Kisoro	1
Influenza virus	Influenza A	Human	Bwindi Community Hospital (Kisoro)	1
Rhabdovirus	PREDICT_RbdV-7	Lesser Short-Nosed Fruit Bat	Bunga	1
	PREDICT_RbdV-8	Unidentified Brush-Furred Rat	Kisoro	1
Picornavirus	Enterovirus J	Vervet Monkey, Olive Baboon	Mukono, Zirobwe, QENP	32
	Rhinovirus C	Olive Baboon	QENP	1
Retrovirus	Baboon foamy virus	Olive Baboon	QENP	8
Adenovirus	PREDICT_AdV-31	Black Rat	Nombe	1
	PREDICT_AdV-32	Natal Multimammate Mouse	Kisoro	1
	PREDICT_AdV-43	Unidentified Brush-Furred Rat	Nkuringo	2
	PREDICT_AdV-47	Unidentified Swamp Rat	BINP	1
	PREDICT_AdV-52	Stella Wood Mouse	BINP	1
Herpesvirus	Gorilla lymphocryptovirus 1	Mountain Gorilla	BIF	61
	Baboon cytomegalovirus/ eye/CII-163	Olive Baboon	QENP	4
	Cercopithecine herpesvirus 5	Vervet Monkey	Mukono	2
	Chlorocebus rhadinovirus 1	Vervet Monkey	Mukono, Nyamusingiri	6
	Gorilla rhadinovirus 1	Mountain Gorilla	BIF	1
	Green monkey lymphocryptovirus/marrow/CII-040	Vervet Monkey	Mukono	3
	Green monkey lymphocryptovirus/marrow/CII-051	Vervet Monkey	Nyamusingiri	1
	Macacine herpesvirus 4/Africa	Vervet Monkey	Mukono	4
	Macacine herpesvirus 4/Asia	Olive Baboon	QENP	1
	Panine herpesvirus 2	Mountain Gorilla	BIF	14
	Papiine herpesvirus 1	Vervet Monkey	Mukono	24
	PREDICT_HV-22	Vervet Monkey	Mukono	2
	PREDICT_HV-40	Vervet Monkey	Mukono	4
	PREDICT_HV-126	Mountain Gorilla	BIF	1
	PREDICT_HV-127	Mountain Gorilla	BIF	1
	PREDICT_HV-37	Unidentified Rhinolophus Bat	MGNP	2
	PREDICT_HV-43	Unidentified Tadarida Bat	QENP	1
	Rattus rhadinovirus 1	Black Rat	Kisoro, Nyanswiga	2
	PREDICT_HV-29	Unidentified Crocidura Shrew, Jackson’s Soft-Furred Mouse	Kisoro, BINP	3
	PREDICT_HV-30	Big-Eared Swamp Rat	BINP	1
	PREDICT_HV-31	Unidentified Brush-Furred Rat, Unidentified Swamp Rat, Unidentified Soft-Furred Mouse	Kisoro	5
	PREDICT_HV-32	Unidentified Brush-Furred Rat	Nkuringo, Nombe	4
	PREDICT_HV-33	Unidentified Brush-Furred Rat, Unidentified Soft-Furred Mouse	Kisoro	3
	PREDICT_HV-34	Stella Wood Mouse, Unidentified Swamp Rat	BINP	2
	PREDICT_HV-35	Big-Eared Swamp Rat, Unidentified Swamp Rat	BINP	2
	PREDICT_HV-36	Unidentified Brush-Furred Rat	Nkuringo	2
	PREDICT_HV-38	African Giant Shrew, Black Rat	Kisoro	2
	PREDICT_HV-39	Unidentified Brush-Furred Rat, Unidentified Swamp Rat	Nkuringo, Nombe, Kisoro, BINP	8
	PREDICT_HV-41	Unidentified Swamp Rat	BINP	1
	PREDICT_HV-42	Unidentified Soft-Furred Mouse	Kisoro	1
	PREDICT_HV-45	African Giant Shrew	Kisoro	1
	PREDICT_HV-49	African Giant Shrew, Unidentified Crocidura Shrew, Natal Multimammate Mouse, Black Rat	Kisoro	5
	PREDICT_HV-50	Unidentified Soft-Furred Mouse	Kisoro	1
Total				**252**

BINP = Bwindi Impenetrable National Park; QENP = Queen Elizabeth National Park; BIF = Bwindi Impenetrable Forest; MGNP = Mgahinga Gorilla National Park

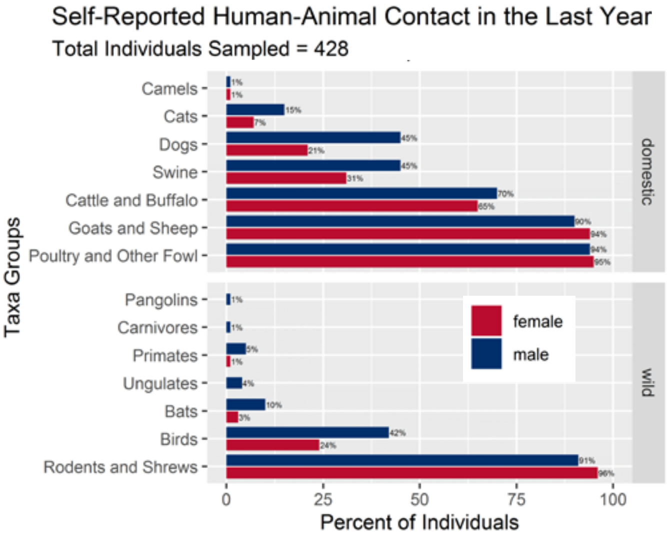
VIRUSES DETECTED IN HUMANS & WILDLIFE

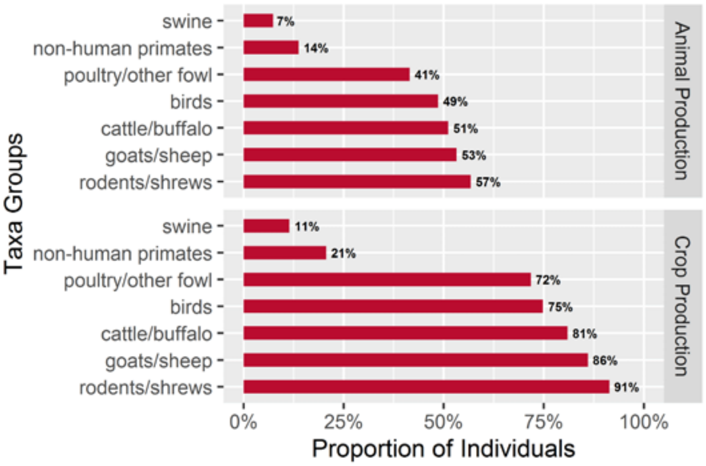
Within PREDICT Uganda from 2009-2019, we detected a total of 59 viruses belonging to a diverse array of virus groups. A majority (39) of these viruses were novel discoveries, while the remaining 20 were known viruses. Rodents and shrews were host to 29 of these viruses, non-human primates hosted 18, bats hosted 11, and one virus was found in a human. The virus infecting a human was a known influenza virus (influenza A) detected in a patient at the Bwindi Community Hospital in Kisoro. Subtyping to distinguish the H and N designations of this virus was recommended to ministry partners.

The most virus diversity was found in the herpesvirus group, where we detected 33 unique viruses (21 novel and 12 known) predominately in non-human primates and rodents/shrews. Coronavirus diversity was second highest, having discovered 10 unique viruses (seven novel, three known) infecting insect-eating bats and several rodents/shrews. A known coronavirus, coronavirus 229E (bat strain), was detected in roundleaf and horseshoe bats within the *Hipposideros* and *Rhinolophus* genera. This particular strain infects bats, and while there is a strain of a known alphacoronavirus that infects humans (human coronavirus 229E), which is known to cause respiratory illness in people, the bat-specific strain we detected is not considered a public health threat. Additionally, coronavirus 229E (bat strain) was the only virus detected during both parts of the project in PREDICT-1 (2009-2014) and PREDICT-2 (2014-2019), but it was detected at different sites and in different bat species. One insect-eating bat, Geoffroy's horseshoe bat (*Rhinolophus clivosus*), was co-infected with two novel coronaviruses, PREDICT_CoV-43 and PREDICT_CoV-65. Neither of these two coronaviruses, nor any other novel coronaviruses discovered, are related to the novel coronavirus SARS-CoV-2, the virus responsible for the current COVID-19 pandemic. All paramyxoviruses, rhabdoviruses, and adenoviruses discovered (12 total) were novel viruses predominately infecting rodents/shrews, as well as two bat species.

EPIDEMIOLOGIC & BEHAVIORAL RISK

Human surveillance conducted at Bwindi Community Hospital included both biological sampling, as described above, as well as qualitative and quantitative behavioral data collection in patients presenting with fevers of unknown origin. From January 2017-July 2018, 413 patients participated in clinic-based surveillance and were administered behavioral questionnaires. Fifteen (15) park staff also completed behavioral questionnaires. Interviews were structured to include questions on demographics, travel histories, knowledge of disease origins, medical history and contact with wild and domestic animals.





EDUCATION & DISEASE AWARENESS

Of patients surveyed who were older than 12, and who reported having had an illness in the past year, 93% (333/358) stated they did not know the cause of their illness or sickness. Less than 2% of respondents stated that their illness came from ingesting bad food or water, being bitten by mosquitos, or from contact with other sick people.

LIVELIHOODS

The predominant primary livelihood of all 428 participants was crop production (94% of females; 82% of males). The second most common livelihood was animal production, with 71% of females and 70% of males reporting raising animals. The most common animals raised were poultry, goats and sheep, and/or pigs. The remaining livelihoods included non-animal businesses and protected area workers.

SYMPTOMS

On average, patients presented to clinics after an average of 6.8 days with fever at the time of sampling. The most common symptoms in addition to fever were: headache (91%), dizziness (70%), loss of appetite (61%), abdominal pain (50%), malaise (50%), and chills (45%).

Patients were categorized in one of five categories for analysis: 1) fever with cough, 2) fever with vomiting/diarrhea, 3) fever with hemorrhaging, 4) fever with encephalitis, and 5) fever with headache. Males were 2.2 times more likely to have fever with cough than females. For the remaining symptom groups, there were no significant differences between genders. No symptom group varied significantly based on age groups of 0-10, 11-20, 21-40, 41-60, and 61+ years of age.

REPORTED WILD ANIMAL CONTACT

Both males (91%) and females (96%) reported that most of their contact with wildlife was with rodents and shrews. Of all who reported contact with rodents, 82% reported finding rodents in and around their home, 77% found rodent feces in or around food, and 58% reported handling live rodents. Both males (42%) and females (24%) reported contact with wild birds, with most respondents reporting handling live wild birds. Few respondents (less than 5%) reported contact with other wildlife, including ungulates (e.g. duiker, buffalo), primates, carnivores, and pangolins.

REPORTED DOMESTIC ANIMAL CONTACT

Most respondents (94% of females and 95% of males) reported contact with poultry. Of those, 88% handled live poultry, 64% cooked or handled poultry raw, 60% found poultry feces around human food, and 66% reported poultry present in their home. Similarly, both males (90%) and females (94%) reported contact with goats and sheep, with 76% respondents reporting raising goats and sheep and 58% reporting cooking or handling raw goat or sheep meat. Both genders (male 70% and female 65%) reported contact with cattle, with the most common contact types being handling live cattle (55%) or cooking or handling raw cattle meat (30%).

LIVELIHOOD-SPECIFIC ANIMAL CONTACT

Both wild and domestic animals were reported raiding food supplies or destroying crops by individuals engaged in animal and crop production. Rodents/shrews were most commonly reported, followed by goats and sheep, cattle, and birds. Individuals also reported raiding crops (21% of respondents) or livestock food supplies (14% of respondents) by non-human primates. Wild birds were also commonly reported to raid or destroy crops (75% of respondents) or animal food supplies (49% of respondents).

DEMOGRAPHICS OF HUMANS INTERVIEWED

		# OF INDIVIDUALS
GENDER	FEMALE	242 (56.5%)
	MALE	186 (43.5%)
AGE GROUP	ADULT (≥18 YRS)	292 (68.2%)
	CHILD	136 (31.8%)
AGE	MEAN (SD)	26.3 (13.1)
	MEDIAN [MIN,MAX]	22.0 [11.0, 73.0]

RISK COMMUNICATION

PREDICT utilized field surveillance activities as a platform for sensitizing local communities around its purpose. Community members and partner organization staff members learned about wildlife as the source of most human emerging infectious pathogens and the routes by which viral spillover occurs. In so doing, PREDICT created opportunities for community members and partners to engage in overall efforts through question and answer sessions. As well, PREDICT initiated local One Health outreach efforts in the greater Bwindi-Mgahinga Conservation Area, using tools developed by the project that convey easily understood, targeted messages to community members on how best to protect themselves and their families from bat-borne pathogens.

As the PREDICT project came to a close in September 2019, our team held a symposium at Bwindi Community Hospital attended by over 100 participants representing the hospital, regional District Health and Veterinary Officers, Uganda Wildlife Authority-Bwindi park personnel, Village Health Teams, Bwindi Nursing School students, and community business leaders. During this meeting PREDICT provided information on project results to its government partners and the communities in which surveillance was conducted. This event was also an opportunity to share and distribute a practical tool, PREDICT's *Living Safely with Bats* behavior change and risk communication resource, which illustrates how communities can live safely with bats and protect themselves and their domestic animals from bat-borne diseases.

PRACTICAL IMPLICATIONS

In a country where multiple deadly viruses have spilled over from wildlife and caused outbreaks (e.g. Ebola, Marburg), and where other pandemic viruses (e.g. West Nile virus, Zika virus) with animal origins have been first detected, Uganda is a living laboratory for the threat of emerging infectious diseases resulting from wildlife-human contact.

PREDICT confirmed that viruses are circulating in wildlife in Uganda that are closely related to viruses known to cause human disease (e.g., a MERS-like coronavirus detected in a bat; see special feature on next page).

Intensive pathogen and behavioral surveillance in southwestern Uganda revealed a high-level of contact among people and wildlife, and therefore the potential for virus sharing, or "spillover."

We detected serologic evidence for exposure to potentially lethal filoviruses (e.g. Ebola virus) in people living in the greater Bwindi Impenetrable Forest area and demonstrated that people who reported contact with forest wildlife (e.g. duiker) were more likely to exhibit antibody titers to filoviruses.

We recommend the continuation of wildlife pathogen surveillance and characterization, comprehensive virus screening in febrile human patients, and further studies to better understand the ecological and behavioral drivers of viral spillover.

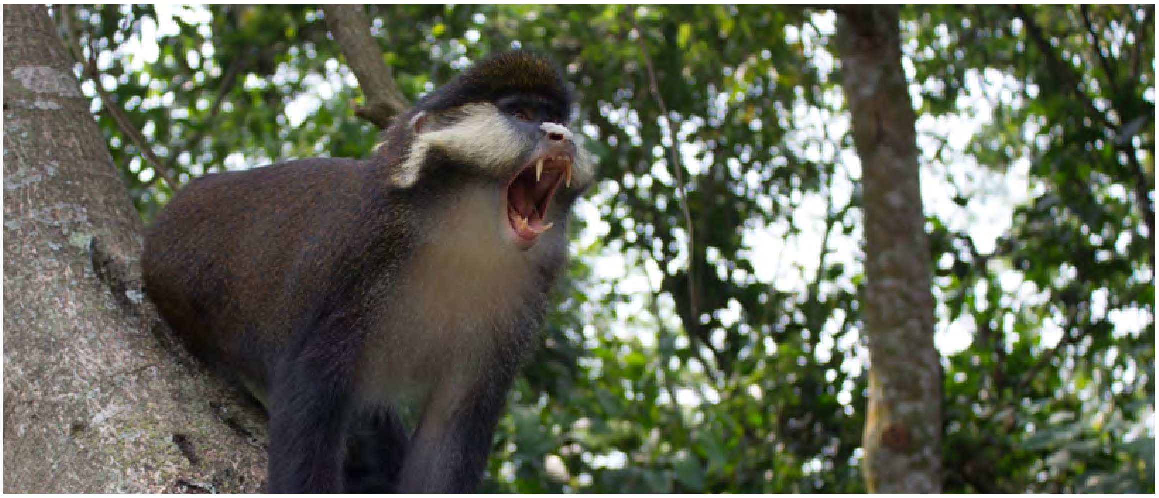
SPECIAL FEATURE

IDENTIFICATION OF A NEW CORONAVIRUS CLOSELY RELATED TO MERS-CoV

During the first phase of the PREDICT project (2009-2014), we detected a new MERS-like coronavirus, PREDICT/PDF-2180 (GenBank accession number KX574227) from an insectivorous bat, *Pipistrellus hesperidus*, bat sampled in Kisoro District in 2013 within our concurrent One Health surveillance area. This virus is closely related to the one that causes Middle Eastern Respiratory Syndrome (MERS). This finding provides further evidence for bats as the potential reservoir for emerging infectious diseases such as MERS, SARS, and COVID-19. To better understand the potential risk posed to human health by this virus, our Uganda team conducted in-depth molecular characterization of PREDICT/PDF-2180 and confirmed that the virus was not likely to pose a threat to human health. This important message was conveyed to government partners and Bwindi Community Hospital.

The open-access publication was published in the scientific journal mBio: **mbio.asm.org/content/8/2/e00373-17**

SPECIAL FEATURE



EVIDENCE OF EXPOSURE TO EBOLAVIRUSES IN KISORO DISTRICT

During the first phase of PREDICT (2009-2014), PREDICT Uganda implemented a separate but aligned research endeavor in partnership with Bwindi Community Hospital, implementing human behavioral surveillance and biological sampling in patients with fevers of unknown origin who reported contact with wildlife. Analyses of data resulting from this focused behavioral and serological survey that were completed during PREDICT-2 (2014-2019) demonstrated exposure to potentially lethal filoviruses (e.g. Ebola virus) in people living around Bwindi Impenetrable National Park. This special study also revealed that people who reported contact with forest wildlife (e.g. duiker – a common antelope species) were more likely to exhibit antibody titers to filoviruses, indicating that hunters and people who consume bushmeat are a particularly at-risk group for filovirus (e.g. Ebola virus) spillover and spread..

Learn more at **bit.ly/p2-uganda-filovirus**

Local market, Dong Thap Province.

PHOTO: WCS

ASIA

PREDICT
BANGLADESH

ONE HEALTH IN ACTION (2009-2020)

10 years of PREDICT Bangladesh has built a cohort of One Health practitioners, deepening the region's capacity

The second phase of the PREDICT project (2015-2020) targeted high-risk sites within geographic 'hot spots' for emerging infectious diseases (areas where a confluence of risk factors may contribute to disease emergence and spread). The PREDICT project focused on wild animals (macaques, bat, rodents) and high-risk communities to enhance and advance the One Health approach to disease surveillance in Bangladesh. In addition to the animal, behavioral risk, and hospital-based syndromic surveillance, PREDICT Bangladesh contributed to numerous outbreak investigations at the request of government partners. Four PREDICT project study sites in Dhaka, Madaripur, Faridpur, and Dinajpur were prioritized by identifying areas considered high-risk for human contact with a diversity of wildlife, many known to be associated with zoonotic virus diversity and with ecological and epidemiological conditions associated with disease emergence.

LOCAL PARTNERS

- Bangladesh Forest Department (BFD)
- Bangladesh Livestock Research Institute (BLRI)
- Chittagong Veterinary and Animal Sciences University (CVASU)
- Department of Livestock Services (DLS)
- Food and Agriculture Organization of the United Nations (FAO) Bangladesh
- International Centre for Diarrhoeal Disease Research, Bangladesh (iccdr,b)
- Institute of Epidemiology, Disease Control and Research (IEDCR)

Global Health Security Agenda

>140

DEVELOPED the One Health Workforce by training more than 140 people in Bangladesh.

>20.3K

OPERATIONALIZED One Health surveillance and sampled over 20.3K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Institute of Epidemiology, Disease Control & Research
- icddr,b

80,090
TESTS

238
PREDICT

39
KNOWN

DETECTED 277 unique viruses in both animal and human populations.

Dr. Ariful Islam is a veterinary epidemiologist and disease ecologist and works as a senior scientist at EcoHealth Alliance. As the Bangladesh Programs Coordinator at EcoHealth Alliance, Dr. Ariful coordinates all of EcoHealth Alliance's surveillance and capacity building efforts in Bangladesh.

Dr. Arif worked for more than a decade as the Bangladesh Country Coordinator for the PREDICT project. He has more than twelve years experience in conducting epidemiological studies, wildlife surveillance for emerging zoonotic disease threats, and behavioral risk characterization in high-risk communities in Bangladesh. His research focuses on understanding the drivers of zoonotic disease emergence and the ecology and evolution of bats and their associated viruses such as Nipah, Ebola, SARS, and Middle East Respiratory Syndrome (MERS) viruses at the animal, human, and ecosystem interface.

Dr. Ariful works closely with partners from the Institute of Epidemiology, Disease Control and Research (IEDCR), the Bangladesh Forest Department, the Department of Livestock Services, Bangladesh Livestock Research Institute (BLRI), the International Centre for Diarrhoeal Disease Research, Bangladesh (iccdr,b), and local universities to develop personnel and laboratory capacity and establish sustainable disease surveillance systems.

The mentorship Dr. Islam received through the PREDICT project has enabled him to tackle the challenges of conducting wildlife surveillance in Bangladesh. Additionally, the close relationships he has established with laboratory partners across the country have broadened Dr. Islam's understanding of molecular diagnostic assays. These honed skills have positioned Dr. Islam in the workforce that is working toward a Bangladesh that is more prepared for emerging disease threats.

ARIFUL ISLAM
Senior Scientest,
EcoHealth Alliance

ACHIEVEMENTS

- The PREDICT Bangladesh team supported One Health emerging disease surveillance by sampling nearly 19,000 animals from over 60 different species.
- During phase two of the PREDICT project, the team significantly improved the country's understanding of virus diversity and circulation, detecting sequences from 40 unique viruses across four virus groups.
- The PREDICT Bangladesh team supported multiple disease outbreak investigations at the request of the government, including multiple crow mortality events, a leptospirosis outbreak, and three human outbreaks of encephalitis of unknown origin.
- The PREDICT Bangladesh team identified varied temporal patterns of seropositivity against viruses in fruit bats suggesting the need to target mitigation activities throughout different times of the year. Additionally, PREDICT Bangladesh found evidence of co-infection in bats. These patterns are based on the dynamics of Nipah virus, filoviruses, and rubulaviruses in a single species of bat, *Pteropus medius*.
- The PREDICT Bangladesh team identified MERS antibodies circulating in dromedary camels that were imported to and born in Bangladesh.
- The PREDICT Bangladesh team supported in-service and pre-service training workshops for local academic and government institutions. PREDICT Bangladesh supported a Master's thesis on the ecology of antimicrobial resistance in wildlife.
- The PREDICT Bangladesh team identified high levels of antimicrobial resistant bacteria in wildlife suggesting that wildlife living in close proximity to human and livestock populations are frequently exposed to resistant bacteria.
- The team conducted detailed genome studies on *Pteropus medius* greatly expanding our understanding of the diversity of viruses in this fruit bat species. Sequences from bat Nipah viruses suggest that the virus changes little during spillover events suggesting that outbreak strains in humans are likely most similar to the strain of Nipah circulating in the local bat population.

ONE HEALTH SURVEILLANCE

Bangladesh has been identified as an emerging infectious disease hotspot where high human and livestock population densities, rapid landscape change, and deforestation have created multi-faceted animal-human interfaces. PREDICT Bangladesh focused on four key locations that represent these critical animal-human interfaces: Madaripur, Dhaka, Dinajpur, and Faridpur.

- **Madaripur District**: A rural-urban site with unique and evolving regulations regarding the local macaque populations. Macaques, once protected and fed by the government, are no longer provisioned with food and are now in direct competition for food and resources with people and livestock living in the area. These changes resulted in a large amount of human-macaque contact that is frequently aggressive in nature. This contact made it an important site to investigate virus sharing between wildlife, livestock, and people.
- **Dhaka District**: As the capital of Bangladesh, Dhaka is an urban site with increasing intensification of animal production to meet the demand of the ever-growing human population. This site was selected to understand whether increased anthropogenic and domestic animal pressure on urban dwelling macaque populations affects the likelihood of aggressive contact with people.
- **Dinajpur District**: This site is located on the border where regular livestock trading occurs between Bangladesh and India. At this site, the PREDICT Bangladesh team coordinated with the FAO to monitor trans-boundary livestock trade routes and target surveillance in areas where wildlife, people, and livestock have frequent contact.
- **Faridpur District**: A rural site along the rural-urban land-use gradient (with Madaripur and Dhaka), where there are high levels of wildlife-human contact. This site also has a known history of Nipah viral spillover events in the local communities.

Within these four locations, human disease surveillance focused on people living in close contact with wildlife, specifically city-dwelling macaques, and on people working within the livestock value chain. Syndromic surveillance of febrile patients was conducted at the Faridpur Medical College Hospital, which served the catchment area in and around the main PREDICT project sampling sites.

The PREDICT Bangladesh One Health surveillance activities established a safe and secure biobank of 108,575 samples from 20,006 individuals. The team collected samples from humans, wildlife, and domestic animals which included whole blood, serum, oral and nasal swabs, urine, and feces.

LAB CAPACITY

IEDCR and icddr,b, the PREDICT partner laboratories in Bangladesh, are now equipped with the full capacity to conduct the complete range of activities required to safely detect priority zoonotic diseases and emerging viral threats. Samples from wildlife, humans, and domestic animals were tested using consensus PCR (cPCR) to screen for five priority virus groups (coronavirus, flavivirus, filovirus and paramyxovirus). Virus findings were confirmed through sequencing and interpreted to better understand the relationship of the detected sequence to those from known animal and human pathogens. Reports of the interrelated results were produced and shared with the One Health Secretariat and local government ministries. This joint approval process supported and catalyzed multi-sectoral linkages, One Health dialogue, information sharing, and collaboration between the animal and human health sectors in Bangladesh. Both laboratories continue to test animal and human samples and serve as key training centers for students and professionals, including government staff from the national laboratory system.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Dhaka, Dinajpur, Faridpur Medical College & Hospital (Faridpur & Madaripur)	7	1	6
	Coronavirus 229E (Human strain)	Human	Madaripur	2	0	2
	Human coronavirus HKU1	Human	Dhaka	4	0	4
	PREDICT_CoV-17	Indian Flying Fox	Bogra, Dhaka, Madaripur	8	4	4
	PREDICT_CoV-24	Greater Short-Nosed Fruit Bat, Lesser Short-Nosed Fruit Bat	Charmuguria, Dinajpur, Gazipur, Madaripur	7	7	0
	PREDICT_CoV-35	Greater Asian House Bat, Lesser Asian House Bat	Dhaka, Dinajpur, Thakurgaon	6	6	0
	PREDICT_CoV-56	Greater Short-Nosed Fruit Bat	Charmuguria, Gazipur	5	5	0
	PREDICT_CoV-68	Indian Flying Fox	Dhaka	1	0	1
	PREDICT_CoV-86	Lesser Bamboo Bat	Benapole	5	5	0
	PREDICT_CoV-88	Lesser Bamboo Bat	Benapole, Madaripur	7	6	1
	PREDICT_CoV-89	Blyth's Horseshoe Bat	Faridpur	1	1	0
	PREDICT_CoV-90	Greater Asian House Bat	Thakurgaon	3	3	0
	PREDICT_CoV-103	Greater False Vampire Bat, Greater Short-Nosed Fruit Bat, Lesser Asian House Bat, Unidentified Megaderma Bat	Benapole, Dinajpur, Faridpur, Madaripur, Sylhet	34	30	4
	Longquan Aa mouse coronavirus	Asian House Shrew, Lesser Bandicoot Rat	Dhaka	3	3	0
	Murine coronavirus	Black Rat, Greater Bandicoot Rat, House Mouse, Lesser Bandicoot Rat, Oriental House Rat, Unidentified Rat	Dhaka, Dinajpur, Madaripur	21	7	14
	Wencheng Sm shrew coronavirus	Asian House Shrew	Dinajpur	6	6	0
	Alphacoronavirus 1 (Canine coronavirus)	Domestic Dog	Dhaka, Pabna Sadar	3	0	3
	Duck coronavirus	Domestic Chicken, Domestic Duck, Domestic Goose, Domestic Muscovy Duck, Domestic Pigeon, Swan Goose	Dhaka, Rajshahi	35	0	35
	Infectious bronchitis virus (IBV)	Domestic Chicken, Domestic Duck, Domestic Goose, Domestic Muscovy Duck, Domestic Pigeon, Common Quail	Dhaka, Dhamrai, Rajshahi	34	0	34
	Pigeon-Dominant coronavirus	Domestic Pigeon	Dhaka, Savar	9	0	9

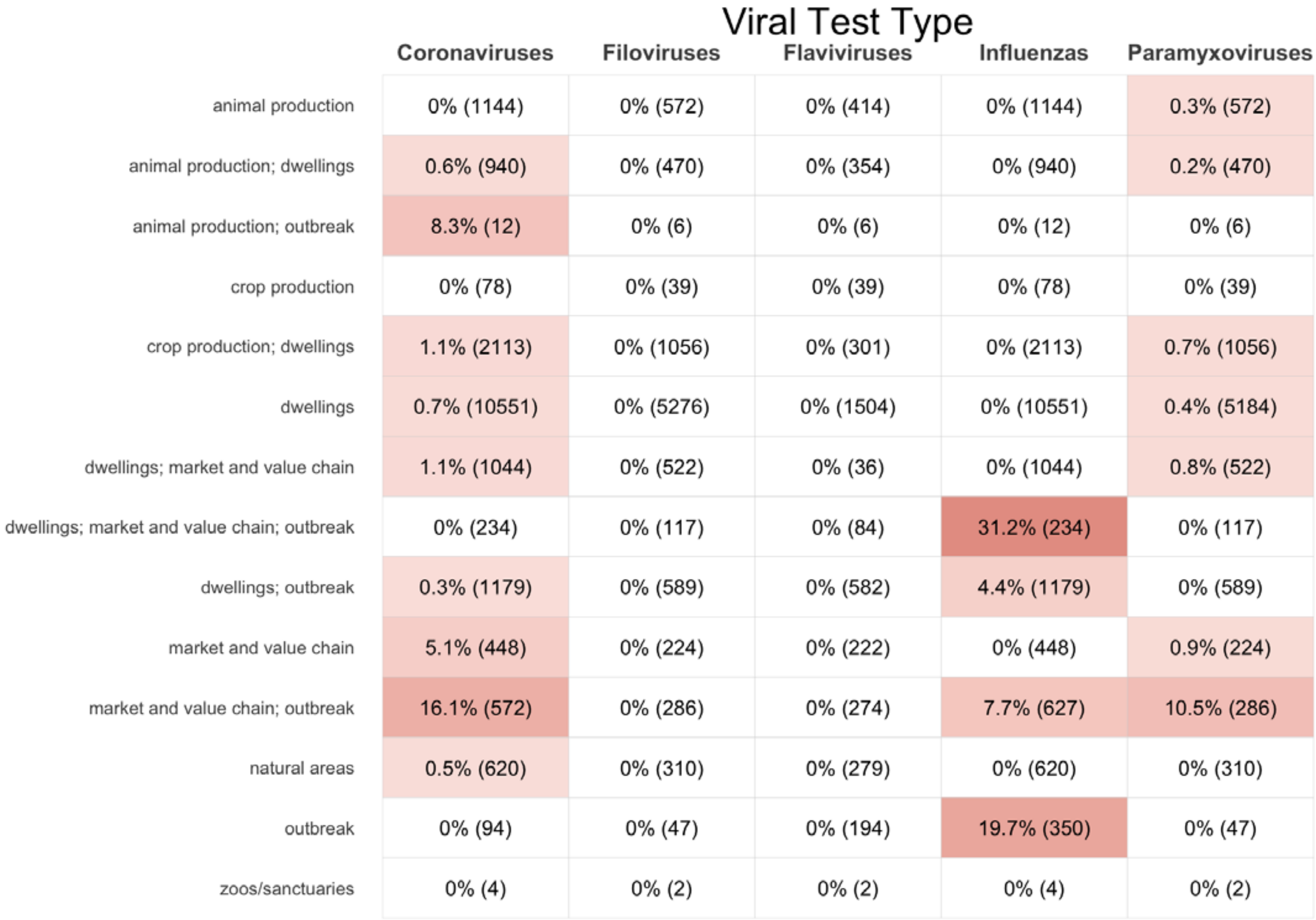
Paramyxovirus	Human parainfluenzavirus 1	Human	Dhaka, Madaripur	3	1	2
	Human parainfluenzavirus 2	Human	Madaripur	1	1	0
	Human parainfluenzavirus 3	Human	Madaripur	1	0	1
	Measles virus	Human	Faridpur Medical College & Hospital (Faridpur & Madaripur), Madaripur	5	1	4
	PREDICT_PMV-13	Lesser Asian House Bat	Dhaka	3	3	0
	PREDICT_PMV-103	Blyth's Horseshoe Bat	Faridpur	1	1	0
	PREDICT_PMV-104	Greater False Vampire Bat	Faridpur	1	1	0
	PREDICT_PMV-109	Naked-Rumped Pouched Bat	Faridpur	1	1	0
	PREDICT_PMV-117	Blyth's Horseshoe Bat	Madaripur	1	0	1
	PREDICT_PMV-20	Asian House Shrew, Black Rat, House Mouse, Unidentified Rat	Charmuguria, Madaripur	16	11	5
	PREDICT_PMV-58	Oriental House Rat, Unidentified Rat	Dinajpur, Madaripur	3	1	2
	PREDICT_PMV-145	Asian House Shrew	Dinajpur	3	3	0
	PREDICT_PMV-149	House Mouse	Dhaka	3	3	0
	Avian paramyxovirus 6	Domestic Chicken, Domestic Duck, Domestic Goose	Dhaka, Rajshahi	7	0	7
	Newcastle disease virus	Domestic Chicken, Domestic Duck, Domestic Goose, Domestic Pigeon	Dhaka, Rajshahi	23	0	23
	Peste des petits ruminants (PPR)	Domestic Sheep	Dhaka	4	4	0
Influenza virus	Influenza A	Human, Common Quail, House Crow, Large-Billed Crow, Domestic Goose, Domestic Pigeon, Domestic Chicken, Domestic Muscovy Duck, Domestic Duck	Dhaka, Dhamrai, Dinajpur, Madaripur, Faridpur Medical College and Hospital (Faridpur & Madaripur), Rajshahi, Savar	182	17	165
	Influenza B	Human	Dhaka, Faridpur Medical College and Hospital (Faridpur & Madaripur), Madaripur	14	8	6
Flavivirus	Dengue virus serotype 2	Human	Dhaka, Faridpur Medical College and Hospital (Faridpur & Madaripur)	6	5	1
	Dengue virus serotype 3	Human	Dhaka, Faridpur Medical College and Hospital (Faridpur & Madaripur)	2	2	0
Total				**481**	**147**	**334**

VIRUSES DETECTED IN ANIMALS

There were over 420 virus detections representing 40 unique viruses from four different virus groups. Over 180 individual animals belonging to four different animal taxa (bats, rodents/shrews, birds, and carnivores) were positive for 17 unique coronaviruses, more than half of which were novel viruses. In addition, we detected Influenza A in over 160 different birds, most of which were from outbreak response. Finally, we detected 12 unique paramyxoviruses in wildlife and domestic animals, nine of which were novel viruses. The novel coronaviruses and paramyxoviruses discovered in wildlife such as bats and rodents/shrews are not believed to pose a threat to human health.

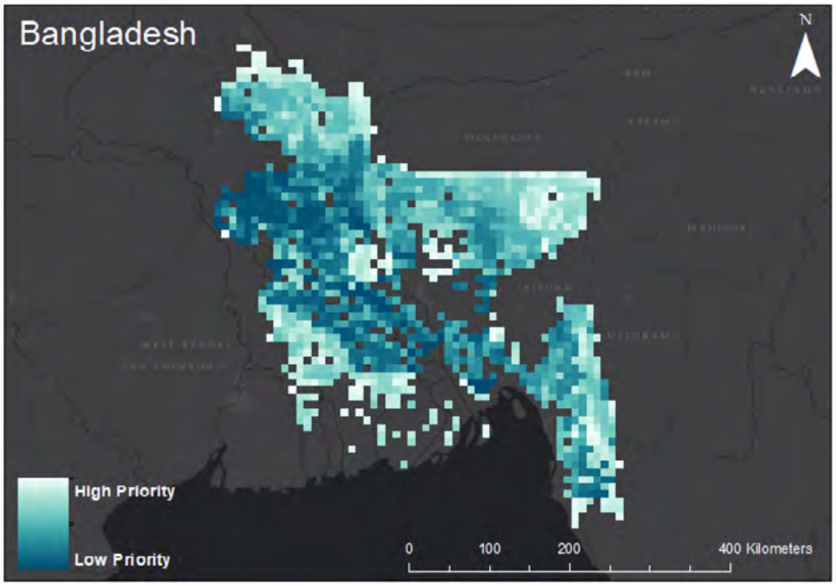
VIRUSES DETECTED IN HUMANS

Ten known viruses representing four different virus groups were detected in humans as a result of community and syndromic surveillance efforts. These include three coronaviruses known to cause respiratory illness in people (229E, OC43, and HKU1), four different paramyxoviruses including three types of human parainfluenza virus as well as measles virus, influenza B, and two serotypes of dengue virus, a known flavivirus transmitted by mosquitoes. Virus summary heatmaps (next page) present the proportion of positive results and the number of PCR tests conducted across the five priority virus groups at key transmission interfaces.



Viral Test Type	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
animal production	0% (1144)	0% (572)	0% (414)	0% (1144)	0.3% (572)
animal production; dwellings	0.6% (940)	0% (470)	0% (354)	0% (940)	0.2% (470)
animal production; outbreak	8.3% (12)	0% (6)	0% (6)	0% (12)	0% (6)
crop production	0% (78)	0% (39)	0% (39)	0% (78)	0% (39)
crop production; dwellings	1.1% (2113)	0% (1056)	0% (301)	0% (2113)	0.7% (1056)
dwellings	0.7% (10551)	0% (5276)	0% (1504)	0% (10551)	0.4% (5184)
dwellings; market and value chain	1.1% (1044)	0% (522)	0% (36)	0% (1044)	0.8% (522)
dwellings; market and value chain; outbreak	0% (234)	0% (117)	0% (84)	31.2% (234)	0% (117)
dwellings; outbreak	0.3% (1179)	0% (589)	0% (582)	4.4% (1179)	0% (589)
market and value chain	5.1% (448)	0% (224)	0% (222)	0% (448)	0.9% (224)
market and value chain; outbreak	16.1% (572)	0% (286)	0% (274)	7.7% (627)	10.5% (286)
natural areas	0.5% (620)	0% (310)	0% (279)	0% (620)	0% (310)
outbreak	0% (94)	0% (47)	0% (194)	19.7% (350)	0% (47)
zoos/sanctuaries	0% (4)	0% (2)	0% (2)	0% (4)	0% (2)

Virus data was integrated with published datasets to support resource allocation decisions for disease surveillance by the Government of Bangladesh. To do this, we combined data on the risk of viral spillover events with data on the relative cost of sampling and access to each site. We predicted optimal areas to target for future surveillance of zoonotic viruses in Bangladesh based on the highest return on investment for sampling. The sites of highest value to sample were ranked from most optimal (white) to least optimal (blue) based on the predicted number of unique zoonotic viruses and the relative cost of sampling and access to each site.



EPIDEMIOLOGICAL & BEHAVIORAL RISK

Bangladesh is a leader in applying the One Health approach to monitoring and mitigating emerging infectious diseases. One of the primary aims of the PREDICT project is to characterize the biological, ecological, and behavioral drivers that facilitate disease emergence and spillover from animals to humans. We performed community-based surveillance, conducting focus group discussions and questionnaires and collecting samples within communities. We also conducted syndromic surveillance in a hospital (Faridpur Medical College & Hospital) serving our primary communities to investigate causes and drivers of acute diseases of unknown origin.

Data indicated that there are high rates of animal contact among the study population across all sampling sites. Of note, participants reported frequent contact with rodents and poultry in the household: 90% of participants had contact with rodents in house, 79% noticed animal feces near food or food preparation areas, and 66% of participants raised poultry as a pet or for sustenance in or near their dwelling and reported cooking or handling poultry.

Known coronaviruses were identified in three patients presenting with influenza-like illness or with fevers of unknown origin (3/250; 1.2%). Interestingly, coronaviruses were detected in ten apparently healthy participants sampled from within the community (10/612; 1.6%). Detection of coronaviruses and/or paramyxoviruses in participants were not associated with age or gender of the participant. However, there was a higher odd of detecting these viruses during the dry season ($p < 0.001$). These results inspired a deeper investigation into the diversity of human coronaviruses found in Bangladesh.

In tandem with the questionnaires, the PREDICT Bangladesh team conducted ethnographic interviews, focus group discussions, and community observations to gain a more in-depth understanding of behavioral risk. The team, composed of practitioners in anthropology, social, and clinical sciences, engaged community members from the four sites in discussions complementary to and expanding upon the topics covered in the questionnaire. From land-use change and perceptions of wildlife encroachment to valuations of risky and protective behaviors, team members encouraged respondents to share and expand upon their lived experiences in one of the world's recognized zoonotic disease hotspots.

A central theme in the semi-structured qualitative guides was the way in which the changing physical environment affected the lives of the respondents. Many of the respondents were long-term, if not lifelong, residents of the local areas, and could speak to years of environmental transformation. There was now more development, including roads, homes, and businesses. With the increased human population density, there was landscape change, including fewer trees, fewer ponds and canals (which had been filled-in), and changes in social dynamics. Among the respondents, there was a general sense that while many of these changes were positive, a price had been paid.

> "I have heard about Nipah. Something related to bats, right? There were date tree on that house we lived previously. At that time Nipah came. People were talking about this disease. So we stopped taking raw date juice."
>
> –40 year old female homemaker

> “[T]he old houses are being replaced by high rising buildings. As Gandaria is a part of Old Dhaka, you know, the traditional houses of old Dhaka…they are being sold to the developers and the developers are making high rise buildings. This is quite a big change. There is a field here, named Dhupkhola ground. It was a big one. But right now that has not remained quite a field. You won't see any field now. What you are seeing right now was not like this before. The stalls you are seeing around this field were not here. There was a walkway instead. People used to walk there in the morning. In the afternoon snacks (chatpati, panipuri) were sold on both sides of the road. I mean the environment was quite different. A wonderful one. Now the whole place has been surrounded by walls. The place has not remained like before. The local boys do not play there anymore like before. That has been kept unused. So that is a huge change. And the old houses has also been replaced by the new ones which is also a noticeable change. This place did not have too much stalls, but now it has new stalls growing like fungus. Different stalls of fast foods, departmental stores...”
>
> –27 year old female service holder

Insights from the respondents also demonstrated the ways in which wildlife such as macaques interacted with the community. Macaques were frequently described entering kitchens and other domestic areas, opening food and water containers, ruffling clothing, and stealing food. Physical contact with the macaques was reported with some regularity, including bites and scratches. Some attributed these macaque-human conflicts to a change in home ranges driven by deforestation. Nevertheless, the perceptions of the community members towards macaques varied, from regarding them as a nuisance and a danger, to a form of entertainment and something to be viewed with humor.

Understanding local practices, attitudes, and gaps in risk perception provided the basis of the questions asked over the course of the interviews. For example, probing on behaviors following outbreaks and the general disposal of dead animals revealed the tendency for many respondents to discard (possibly infected) carcasses into nearby ponds and rivers. The interviews suggest that a majority of participants visit local medical facilities when they have health concerns. However, certain individuals still seek out traditional medicine instead. The respondents' perceptions around Nipah virus and avian influenza virus were often inaccurate or incomplete, suggesting that previous educational campaigns had varying levels of success and thus identified potential areas for improvement in future risk communication initiatives.

The scope of this immense qualitative dataset was an achievement made only possible by the skill, experience, and tenacity of the transdisciplinary PREDICT Bangladesh team. Covering diverse geographic regions, socioeconomic categories, gender, ages, and occupations, the data and findings stemming from this investigation will provide valuable resources for researchers and practitioners looking to build locally relevant, evidence-based interventions.

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

The PREDICT Bangladesh team was committed to raising awareness and improving knowledge of zoonotic disease threats while strengthening capacity for disease prevention and response at all levels. This required multi-faceted stakeholder engagement beginning at the community level. Prior to the initiation of sampling, community outreach events were held to introduce the project to the community and raise people's awareness of zoonoses and high-risk animal-human disease transmission interfaces. This community interaction is key to preventing outbreaks of zoonotic disease. The team engaged local communities to increase zoonotic disease awareness and sensitize them to the importance of One Health approaches. The PREDICT Bangladesh team supported zoonotic disease sensitization through the *Living Safely with Bats* tool, a community resource developed by the PREDICT project.

The team continued to work with district and regional level animal and human health officers to transfer knowledge and advance disease surveillance skills. Findings were shared with One Health platform stakeholders to foster engagement at national and community levels and improve awareness of zoonotic diseases and transmission pathways, along with potential prevention and control strategies.

CAPACITY STRENGTHENING

The PREDICT Bangladesh team supported government partners and the One Health Secretariat as the Government of Bangladesh expanded the national One Health platform. Through meetings, workshops, conferences, and in-service and pre-service training opportunities, the team contributed to the One Health framework and strengthened One Health workforce capacity in Bangladesh. PREDICT Bangladesh worked closely with government partners, hospitals, and universities to successfully operationalize the One Health approach and further empower the national One Health platform. PREDICT project partnerships were built to support the One Health mission of the Government of Bangladesh, specifically through wildlife disease surveillance, disease outbreak investigation and response, human behavioral risk investigation, and syndromic surveillance. Additionally, data platforms were strengthened to improve the dissemination of animal and human epidemiological and ecological data, building capacity for zoonotic disease surveillance.

The PREDICT project enabled the surveillance of pathogens that could spillover from animal hosts to people by strengthening capacities to detect viruses circulating in wildlife and domestic animals. The PREDICT Bangladesh team organized over 10 multi-day intensive field-training events for the Government of Bangladesh staff, including forest, BLRI, DLS, and IEDCR officers. The workshops included training on zoonotic diseases, safe sampling methodology, human and animal safety during wildlife capture, personal protective equipment (PPE) use during field sampling and laboratory testing, biosafety and zoonoses, outbreak response data collection and management, and laboratory methodology and safety. Laboratory training and capacity building was a specific focus to advance the portfolio of the internationally recognized work of icddr,b. This included improved capacity for virus discovery and surveillance to monitor spillover of viruses within key virus groups (paramyxoviruses, coronaviruses, filoviruses, flaviviruses, and influenza viruses), from wildlife and livestock populations into human populations.

The PREDICT Bangladesh team supported the government's mission to promote the health and welfare of all species, a One Health approach. The PREDICT Country Coordinator and subject matter experts supported the One Health Secretariat in building the national ability to monitor viral threats; investigate viruses with pandemic potential at high-risk interfaces; build the capacity for the detection and characterization of novel wildlife viruses; improve the understanding of the dynamics of zoonotic viral spillover, evolution, amplification, and spread in order to inform prevention strategies; and reduce the risk of disease emergence. The PREDICT team also supported the Government of Bangladesh's mission to build an outbreak response team through the many scenario-based, joint outbreak investigation training events led by the PREDICT Bangladesh team, and by supporting on-the-job training during disease outbreak investigations. PREDICT Bangladesh supported the growth of the national One Health platform (through the One Health Secretariat) by increasing sub-national, national, and international networks; supporting strategic planning and disease prioritization; participating in health advocacy and communication; and facilitating information sharing between ministries.

The PREDICT Bangladesh team supported One Health platform activities by leading workshops in collaboration with FAO Bangladesh at the National One Health Meetings and OHSA conferences. The PREDICT Bangladesh team built regional and international partnerships within the One Health community. These partnerships were key for sharing knowledge, information, and data. To this end, the PREDICT Bangladesh team worked with the PREDICT India team to capture, sample, and safely release wildlife, and train them in the use of personal protective equipment. These collaborative, cross-border activities have enhanced the workforce capacity to conduct wildlife disease surveillance in India. The PREDICT Bangladesh team presented at a local PREDICT Nepal meeting to share challenges and success stories in order to create collaborative opportunities at a regional level. Simultaneously, the PREDICT Bangladesh team continued to build a network of One Health professionals through an increased presence within the international scientific community by participating in and presenting research findings at numerous international conferences.

PRACTICAL IMPLICATIONS

- The PREDICT project used a collaborative One Health approach to build capacity for the detection and characterization of novel wildlife viruses and to improve the understanding of the dynamics of zoonotic viral spillover, evolution, amplification, and spread in order to inform prevention strategies and reduce the risk of disease emergence.
- The PREDICT Bangladesh team identified important interfaces for disease transmission from animals to humans, including landscape-level changes, which were often associated with deforestation and changing agriculture and food production systems; wildlife hunting; transboundary animal trading; and unplanned commercial farming. These interfaces provide opportunities for pathogens to spillover from animals to humans, so identification is key for risk assessment and resource allocation.
- The support and training that the PREDICT Bangladesh team provided in zoonotic outbreak response has improved the capacity for the Government of Bangladesh to mount a One Health response to outbreaks.
- The team's economics study has the potential to inform country action as well as encourage wider multi-sectoral accounting of disease outbreaks. These results will inform One Health approaches to emerging and endemic zoonotic disease management in the region and world.

SPECIAL FEATURES

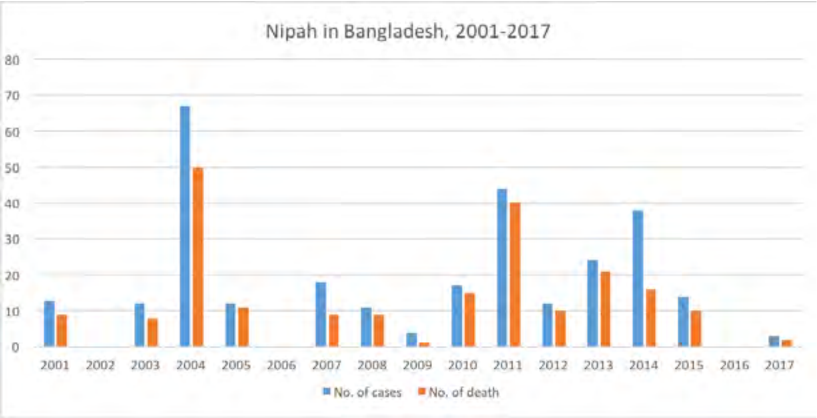
NIPAH IN BANGLADESH

When epidemics become endemic ~ excerpts from an article published in the One Health Communicator, 2018

In Bangladesh, Nipah virus (NiV) causes seasonal encephalitis outbreaks during the winter months (November-April) and so far, cases have only been seen in the western part of the country – the "Nipah belt." Nipah virus outbreaks in Bangladesh on average have a 70% case fatality rate. The virus is a zoonotic paramyxovirus, carried by frugivorous bats throughout Asia. The virus doesn't make bats sick, but people and domestic animals get infected when they eat or drink food that has been contaminated by bat saliva or urine. Nipah virus can be spread from person to person, but not very efficiently, which is what has so far prevented larger epidemics. However, given its broad distribution, high mortality rate, lack of a vaccine or therapeutic, and continual spillover into people in one of the most populous regions on Earth, there is concern that a genetic strain may eventually spillover from bats into people that is more easily transmitted.

SWEET BUT RISKY DELICACY IN BANGLADESH

Drinking raw date palm sap during the winter season is a cherished custom in Bangladesh. The sap is harvested by shaving the bark from the upper trunk of date palm trees and allowing the sap to flow along the trunk into a clay collection pot overnight. Fruit bats, which also eat nectar, have learned to exploit this otherwise unavailable food by landing on the palm fronds and licking the sap as it flows, contaminating the sap with saliva and other excreta, such as when the bats urinate over the collecting pots. Most of the Nipah virus outbreaks in Bangladesh have been associated with drinking raw date palm sap. From 2001-2017, altogether 289 people were infected in Bangladesh among which 211 died. Survivors frequently suffer from prolonged nervous system disorders including muscular weakness, cognitive impairment, and personality change.



Hunting bats for human consumption, living nearby and under bat roosts, and sharing food resources could all result in NiV transmission. Furthermore, the risk of bat-borne zoonotic disease transmission encompasses complex, time-varying interactions between humans and their environment that are often driven by culture, climate, and economic development. Therefore, preventing bat-to-human transmission of NiV in Bangladesh is a public health priority. In 2007, researchers persuaded the sap collectors, Gacchi, to cover the trunk of the date palm with a bamboo skirt (Bana), which prevents the bats from contaminating the sap. Some other effective preventive measures include broadcasting awareness messages on local TV, developing posters, increasing access to handwashing stations in hospitals, and reducing caregivers' exposure to infected patients' bodily secretions during care and traditional burial practices. Most importantly reducing human consumption of raw sap could reduce viral transmission and risk of disease emergence.

COEXISTING SAFELY WITH BATS

Despite the zoonotic disease risk, bats are vital to our ecosystem because they pollinate flowers, disperse seeds, and eat insects. Therefore, it is imperative that we find a way to live safely with bats. This is especially important as most outbreaks of bat-borne zoonotic diseases are a consequence of human activities. As these tropical forest habitats for Nipah-carrying fruit bats have been converted into agricultural lands, the bats have sought out other sources of food. The bats have moved into areas that are closer to human dwellings and therefore present a higher risk of zoonotic disease transmission. Thus, we must coexist safely with bats as suggested in the *Living Safely with Bats* resource.

PREDICT
CAMBODIA

ONE HEALTH IN ACTION (2009-2020)

Improving the capacity to detect known and emerging viruses in Cambodia through a One Health approach that includes training of in-service government partners from the human health, animal health, and forestry sectors, as well as students, to perform field surveillance, laboratory testing, data interpretation, and analyses.

During the first phase of the PREDICT project (PREDICT-1; 2009-2014) 3,887 wild animals were safely and humanely sampled including non-human primates, rodents, and bats. We targeted diverse wildlife-human interfaces for surveillance including wildlife hunting and trade; crop raiding by wild animals; wildlife rescue centers; markets and restaurants selling wildlife; bat guano farms; religious and ecotourism sites; and rodents traded to Viet Nam for food to get a better understanding of where risk for spillover of viruses from wild animals to humans was occurring. Samples were safely tested via polymerase chain reaction (PCR) for 16 virus groups at Institut Pasteur du Cambodge (IPC), resulting in the detection of 18 known and 29 novel viruses.

During the second phase of the PREDICT project (PREDICT-2; 2015-2019), surveillance was focused on two high-risk interfaces based on knowledge gained during PREDICT-1. The PREDICT Cambodia team targeted intensifying animal production in the bat guano trade in Kampong Cham Province, and the market value chain of the cross-border rodent trade to Viet Nam in Kandal province. We performed concurrent longitudinal surveillance of people, their animals, and wildlife at these communities and surveyed people who were sampled to better understand risk for viral spillover and to inform on options for behavioral change to decrease risk. We also performed syndromic surveillance of febrile patients who enrolled at any of the three clinics that serve these two high-risk communities.

Virus testing was performed at IPC for seven virus groups with concurrent training and transferring of PREDICT project protocols to the national animal and human laboratories. During PREDICT-2, samples were safely collected from 1,803 people, 2,715 domestic animals, and 5,890 wild animals, including 875 rodents and 5,006 bats. This work resulted in the detection of 19 known and 17 novel PREDICT project-discovered viruses. Of these, two known and four novel PREDICT project viruses were previously detected in PREDICT-1. In total, the PREDICT team in Cambodia detected 35 known viruses and 42 novel viruses throughout the ten year period, for a total of 77 unique viruses.

Viruses with zoonotic potential and those that can affect the productivity and health of livestock were identified in animals. Full genome sequencing was performed to further characterize seven of these viruses. Our testing led to the detection of viruses not routinely tested for in people presenting with Influenza Like Illness (ILI), Severe Acute Respiratory Illness (SARI), Fever of Unknown Origin (FUO), hemorrhagic fever and/or encephalitis in Cambodia, thus expanding the understanding of viruses circulating in people.

The second phase of the PREDICT project also supported One Health initiatives for effective collaboration across disciplines and government sectors, including engagement with the Cambodian Zoonotic Technical Working Group and veterinary and human health university faculties. Through analysis of project data and findings, the PREDICT team was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

IMPLEMENTING PARTNERS

- University of California, Davis
- Institut Pasteur du Cambodge
- Ministry of Health: Department of Communicable Disease Control (CDC)
- Ministry of Agriculture and Forestry: General Directorate of Animal Health and Production (GDAHP)
- Ministry of Agriculture and Forestry: National Animal Health and Production Research Institute (NAHPRI)
- Ministry of Agriculture and Forestry: Forestry Administrations' Department of Wildlife and Biodiversity (DWB)
- Food and Agricultural Organization (FAO)
- Wildlife Conservation Society (WCS)
- USAID

OTHER KEY LOCAL PARTNERS

- National Institute of Public Health (NIPH)
- Royal University of Agriculture (RUA)
- University of Health Sciences (UHS)
- Royal University of Phnom Penh (RUPP)
- Kaoh Thum Referral Hospital
- Kunthabopha Hospital
- Two hospitals in Prey Chhor District
- Kang Meas District veterinarian & veterinarian health workers
- Kaoh Thum District veterinarian & veterinarian health workers
- Chiefs of local villages in Kaoh Thum and Kang Meas Districts

300

DEVELOPED the One Health Workforce by training 300 people in Cambodia.

>14.1K

OPERATIONALIZED One Health surveillance and sampled over 5.7K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Institut Pasteur du Cambodge

79,535
TESTS

42 PREDICT **35** KNOWN

DETECTED 77 unique viruses in both animal and human populations.

VEASNA DUONG

Senior Research Associate, *Institut Pasteur du Cambodge*

"I believe PREDICT has brought many benefits to Cambodia, especially through the laboratory protocols to identify known and newly emerging viruses, through protocols for handling and sampling animals in the field, and in the training of government and students in those protocols. Also, PREDICT has benefitted communities we work with by bringing them knowledge of how they can lower their risk of contracting zoonotic disease both from the wild animals they live closely with and from other animals, too."

VIBOL HUL

Field Coordinator & Senior Laboratory Technician, *Institut Pasteur du Cambodge*

"In the 10 years I've worked on PREDICT, I've learned how to address challenges in the laboratory and also in our field work related to animal handling, including wildlife and domestic animals, cold chain, biosafety and biosecurity. Using PREDICT's surveillance results, I also have the opportunity to share my knowledge of zoonotic diseases with the community, students and government staff. Those experiences have inspired me to pursue a Ph.D. in France on Arenavirus in rodents later this year."

ACHIEVEMENTS

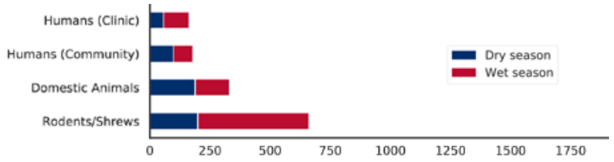
- PREDICT-2 performed the first ever concurrent sampling of wildlife, domestic animals, and people in Cambodia, with excellent collaboration from the human, animal, and forestry sectors of the government. These multi-disciplinary teams were trained in One Health implementation from the field to the laboratory.
- Thirty-three (33) students from the schools of veterinary medicine (8), medicine (2) and epidemiology (23), 13 of whom were women, were trained in PREDICT project laboratory and field sampling protocols. Two of these students have since been hired as permanent staff at Institut Pasteur du Cambodge, and one has received a master's program scholarship for the One Health-focused InterRisk program at Kasetsart University in Thailand (**www.onehealthsea.org/interrisk**). These personal successes reflect the significance and workforce impacts of the project in Cambodia.
- PREDICT project PCR protocols have been adopted for use in outbreaks by the Cambodian government. Two examples include the detection of influenza A (subtyped as H5N1) in a hairy-nosed otter at the Phnom Tamao Zoo, and the detection of Ursid gammaherpes virus after a disease outbreak in bears, also at the zoo.
- The PREDICT team in Cambodia was able to detect SARS-CoV-2, the virus causing the current COVID-19 pandemic, early-on in the outbreak by using PREDICT project test protocols already in use before the SARS-CoV-2 genome was available and before the development of SARS-CoV-2 specific real-time PCR assays.

ONE HEALTH SURVEILLANCE

The PREDICT project's One Health surveillance approach was designed to strengthen capacity for detection of emerging viral threats and to improve our understanding of risk of zoonotic diseases in communities with close and frequent animal contact. The PREDICT team performed the first ever concurrent sampling of wildlife (bats and rodents), domestic animals, and people in Cambodia, targeting high risk populations for viral spillover, amplification, and spread. The team sampled at a bat guano farming community and at an international rodent trade hub. Longitudinal surveillance was conducted over a four-year period during both the rainy and dry seasons, with multi-disciplinary teams from the human and animal health and forestry sectors receiving trainings in One Health approach implementation from the field to the laboratory. Species identification of bats and rodents was confirmed using DNA barcoding, a molecular-based laboratory assay. Surveys of humans were performed at the time of sampling, to better understand behaviors that may increase risk of viral spillover from wild animals to people. The team also conducted syndromic surveillance of patients presenting to local clinics serving the communities at our sites.

KAOH THUM DISTRICT

RODENT TRADE HUB



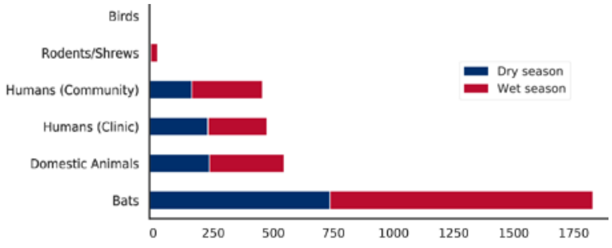
Numbers of individuals sampled by taxa

A potential high-risk market value chain for disease spillover and spread is the cross-border rodent trade from Cambodia to Viet Nam, and was therefore selected as a surveillance site. Rodents are trapped across Cambodia and brought, live and in overcrowded conditions, by middlemen to the rodent trade hub site in Kaoh Thum District, Kandal Province where they are sorted before being transported across the border to Viet Nam to be sold for food. Thousands of rodents a week are traded in the peak season. Sampling targeted the captured rodents, domestic animals at and around the trade hub, and people (trappers, drivers, middle men, children assisting with handling). We also conducted behavioral surveys to explore the practices and behaviors of these individuals that have the potential to facilitate spillover and spread of zoonotic viruses.

Syndromic surveillance was also conducted at a clinic within Kaoh Thum District that serves the local community.

KANG MEAS DISTRICT

BAT GUANO FARMING COMMUNITY



Numbers of individuals sampled by taxa

Our team conducted sampling in rural communities within Kang Meas District, Kampong Cham Province, where construction of artificial bat roosts for the collection of bat guano is common. Roosts are constructed in close proximity to peoples' homes, their children, livestock, and the crops they raise to support their livelihoods. Sampling targeted wildlife (bats and rodents), livestock (coordinated with FAO), and people (farmers, their family, guano middlemen). We also conducted behavioral surveys to explore the practices and behaviors of these individuals that have the potential to facilitate spillover and spread of zoonotic viruses.

In addition to the community surveillance, syndromic surveillance was performed at two clinics serving the local communities, located in Prey Chhor District.

VIRUS DETECTION

The testing strategy in Cambodia for virus detection included screening animal and human samples using broadly reactive consensus PCR (cPCR) for seven virus groups including coronaviruses, filoviruses, flaviviruses, hantaviruses, influenza, paramyxoviruses, and rhabdoviruses.

VIRUS FINDINGS IN PEOPLE

Of the 650 patients sampled at hospitals, 23 were positive for at least one virus. Fifteen people were positive for influenza A, four for influenza B, one for human coronavirus (HKU1), and three for mumps virus. Except for influenza A, none of these viruses are routinely tested for or would have been detected through the routine national surveillance system.

VIRUS FINDINGS IN ANIMALS

Seventeen viruses were detected in bats of which 16 were novel (four coronaviruses, four paramyxoviruses, and eight rhabdoviruses) and one was a known coronavirus. Six known viruses were detected in rodents including two hantaviruses and four coronaviruses. Of importance was the detection of the hantavirus, Seoul virus, in rodents within both districts, as this known zoonotic pathogen can cause respiratory and hemorrhagic illness in people. Eight viruses were detected in domestic animals including one novel coronavirus and seven known viruses (four coronaviruses, one influenza virus, one paramyxovirus, and one flavivirus). The detection of infectious bronchitis virus, influenza A, Newcastle disease virus, and Tembusu virus in domestic animals were noteworthy, as these are viruses are known to affect the health and productivity of these animals.

VIRUS TABLE

				# OF POSITIVE INDIVIDUALS		
VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	TOTAL	WET SEASON	DRY SEASON
Coronavirus	Human coronavirus HKU1	Human	Hospital 1 (Prey Chhor District)	1	0	1
	PREDICT_CoV-24	Greater Short-Nosed Fruit Bat	Kang Meas District	2	1	1
	PREDICT_CoV-25	Lesser Asian House Bat	Kang Meas District	1	1	0
	PREDICT_CoV-35	Lesser Asian House Bat	Kang Meas District	4	2	2
	PREDICT_CoV-56	Greater Short-Nosed Fruit Bat	Kang Meas District	2	1	1
	Bat coronavirus 512/2005	Lesser Asian House Bat	Kang Meas District	83	43	40
	Longquan Aa mouse coronavirus	Greater Bandicoot Rat, Ricefield Rat, Lesser Ricefield Rat, Unidentified Rattus Rat	Kaoh Thum District	70	36	34
	Murine coronavirus	Greater Bandicoot Rat, Ricefield Rat, Lesser Ricefield Rat, Ricefield Ricefield Rat, Ricefield	Kaoh Thum District	190	113	77
	Rodent coronavirus	Ricefield Rat, Ricefield Mouse, Ricefield Rat	Kaoh Thum District	6	3	3
	PREDICT_CoV-100	Domestic Chicken	Kaoh Thum District, Kang Meas District	41	20	21
	Betacoronavirus 1 (Bovine CoV)	Domestic Cow	Kang Meas District	1	0	1
	Betacoronavirus 1 (Canine strain)	Domestic Dog	Kaoh Thum District	1	1	0
	Duck coronavirus	Domestic Chicken	Kaoh Thum District	8	8	0
	Infectious bronchitis virus	Domestic Chicken, Domestic Duck	Kaoh Thum District, Kang Meas District	92	72	20
Paramyxovirus	Human parainfluenzavirus 1	Human	Kunthabopha Hospital	2	0	2
	Human parainfluenzavirus 3	Human	Kunthabopha Hospital	1	0	1
	Mumps virus	Human	Hospital 1 (Prey Chhor District)	3	1	2
	PREDICT_PMV-13	Lesser Asian House Bat, Whiskered Myotis	Kang Meas District	92	66	26
	PREDICT_PMV-63	Lesser Asian House Bat	Kang Meas District	6	4	2
	PREDICT_PMV-66	Lesser Asian House Bat	Kang Meas District	31	23	8
	PREDICT_PMV-67	Lesser Asian House Bat	Kang Meas District	19	17	2
	Newcastle disease virus	Domestic Chicken	Kaoh Thum District, Kang Meas District	3	2	1
Influenza virus	Influenza A	Human, Domestic Chicken, Domestic Duck	Kaoh Thum District, Kang Meas District, Hospitals 1 & 2 (Prey Chhor District)	25	15	10
	Influenza B	Human	Hospitals 1 & 2 (Prey Chhor District)	5	4	1
Flavivirus	Tembusu virus	Domestic Chicken	Kang Meas District	4	2	2
Rhabdovirus	PREDICT_RbdV-21	Lesser Asian House Bat	Kang Meas District	14	8	6
	PREDICT_RbdV-27	Lesser Asian House Bat	Kang Meas District	20	3	17
	PREDICT_RbdV-28	Lesser Asian House Bat	Kang Meas District	7	6	1
	PREDICT_RbdV-31	Lesser Asian House Bat	Kang Meas District	2	2	0
	PREDICT_RbdV-32	Lesser Asian House Bat	Kang Meas District	1	1	0
	PREDICT_RbdV-33	Lesser Asian House Bat	Kang Meas District	1	1	0
	PREDICT_RbdV-34	Lesser Asian House Bat	Kang Meas District	3	3	0
	PREDICT_RbdV-35	Lesser Asian House Bat	Kang Meas District	1	0	1
Hantavirus	Seoul virus	Ricefield Rat	Kaoh Thum District	2	1	1
	Thottapalayam virus	Asian House Shrew	Kang Meas District	2	2	0
Total				**746**	**462**	**284**

EPIDEMIOLOGIC & BEHAVIORAL RISK

Our PREDICT team partnered with local communities to learn more about people's perceptions of risk for exposure to zoonotic diseases and how the types of activities and contact they engage in might influence the risk of disease exposure. Surveys included questions on human demographics, livelihood activities, animal contact, and food safety and sanitation practices. A total of 893 individuals were enrolled in the community-based surveillance – 565 from the bat guano farming community in Kang Meas District and 328 from the rodent trade site in Kaoh Thum District. An additional 836 individuals were enrolled through the syndromic surveillance conducted at the three clinics serving the two communities. Participants were enrolled throughout the year across the rainy and dry seasons, with surveys and sampling occurring concurrently after obtaining informed consent.

Data from the questionnaires identified behaviors and household characteristics that could increase risk of viral spillover from animals to humans in both villages.

Within the Kang Meas District community, 33% of people obtain their drinking water from an uncovered source such as a rainwater cistern, well or pond. At the more developed Kaoh Thum District site, this number was lower at 13%. Such open water sources are not protected from contamination by bat or bird feces or urine from animals living above them, which could contain infectious material. Furthermore, 59% percent of people surveyed in Kang Meas District and 18% in Kaoh Thum District reported sharing their drinking water source with animals. Risky behaviors associated with food consumption included eating food contaminated by animal feces (24% of people in Kaoh Thum District and 27% in Kang Meas District).

People in both communities have livelihoods that lead to a higher than usual contact with bats or rodents, but they reported (and were observed) using limited personal protection while handling bats or rodents, and their feces and urine. A quarter of respondents working in the rodent trade and more than half working on bat guano farms reported wearing no protective clothing. Of those that described wearing some protective gear, a simple hat or cloth gloves were the most common items worn. No one reported using adequate personal protective equipment to reduce risk for disease exposure.

The questionnaire captured participants' lack of awareness of potential disease transmission routes from animals to humans as 84% of respondents in Kaoh Thum District and 72% in Kang Meas District thought there was no health risk or didn't know of any risk from animal bites (Figure 4). Thus, community members did not seek adequate medical treatment when bites or injuries occurred while handling animals. Most (56% in Kaoh Thum District and 50% in Kang Meas District) did nothing following a bite, including not cleaning a wound with water.

	CLINIC SURVEY PARTICIPANTS			COMMUNITY SURVEY PARTICIPANTS	
SITES	HOSPITAL Kaoh Thum District (n=294)	HOSPITAL 1 Prey Chhor District* (n=225)	HOSPITAL 2 Prey Chhor District* (n=317)	Kaoh Thum District (n=328)	Kang Meas District (n=565)
GENDER					
FEMALE	147 (50%)	102 (45.3%)	186 (48.6%)	203 (61.9%)	346 (61.2%)
MALE	147 (50%)	123 (54.7%)	131 (51.4%)	125 (38.1%)	219 (38.8%)

*Hospitals in Prey Chhor District served the community members of Kang Meas District

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

Throughout the project, we held meetings with our partnering communities and with the clinics that participated in syndromic surveillance to discuss how and why the PREDICT project was implemented in Cambodia, shared testing results from human, domestic animal, and wildlife samples, and informed on risky behaviors identified through questionnaires. Some of these behaviors included sharing of drinking water sources and food with animals, eating food contaminated with animal feces, lack of use of Personal Protective Equipment (PPE) when handling animals and their guano, and not understanding the risks from infection following animal bites. We discussed how community members could reduce the risk of exposure to diseases from animals and used the *Living Safely With Bats* book (translated into Khmer), a resource developed by the PREDICT project, as a tool to discuss how to reduce risk.

The communities greatly appreciated these meetings and the opportunity to learn about our testing and questionnaire results. Individuals were very interested in the virus findings, particularly about the viruses that pose a risk to their health or that of their animals. Copies of *Living Safely with Bats* were left with the chiefs of the villages, the local veterinarians, bat farmers, and in libraries at the pagodas and elementary schools so that information could be more widely shared throughout the communities.

Doctors and nurses from the clinics that serve the communities also valued discussion of the virus findings and questionnaires, such as the detection of the zoonotic Seoul virus in rodents, and of the contact patients reported with animals, which could lead to an increased risk of exposure to disease. The *Living Safely With Bats* book was seen as a valuable tool to educate on disease prevention, and doctors requested an electronic version of the book to be produced for use in the waiting rooms at their clinics.

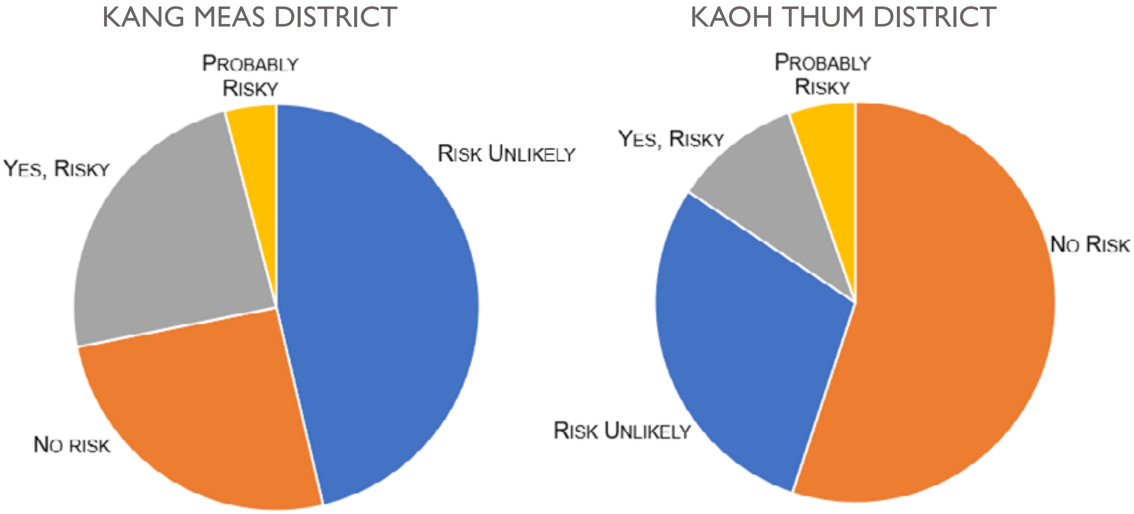


FIGURE 4. ***Perception of Whether Animal Bites Pose a Risk For Disease Transmission in Kang Meas District (top) and Kaoh Thum District (bottom)***

STRENGTHENING CAPACITY

One Health capacity building was a key focus of the PREDICT project and we conducted trainings in the classroom, field, and laboratory for students and in-service government staff from multiple sectors across the One Health spectrum. Trainings developed skills in biosafety and PPE use, safe capture and sampling of wildlife and domestic animals, specimen handling and maintenance of a cold chain, virus detection using consensus PCR, and disease outbreak response.

Twenty-five Cambodian government staff participated in PREDICT project trainings, many of whom also participated in concurrent sampling.

Training from the field to the laboratory was provided to government staff from the Cambodian CDC, the Forestry Administration and the National Animal Health and Production Institute (NAHPRI). Repeat training for government counterparts on safe animal capture and sampling was conducted as part of the project's close-out, to facilitate sustainability of activities beyond the end of the project. Staff from the government's animal and human health laboratories (NAHPRI and the National Institute for Public Health (NIPH)), were trained at the Institut Pasteur du Cambodge to use PREDICT protocols for broadly reactive consensus PCR as a tool to detect both known and unknown viruses.

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- Lacroix, Audrey, et al. "Diversity of bat astroviruses in Lao PDR and Cambodia." *Infection, Genetics, and Evolution* 47 (2017): 41-50. DOI: 10.1016/j.meegid.2016.11.013
- Lacroix, Audrey, et al. "Genetic diversity of coronaviruses in bats in Lao PDR and Cambodia." *Infection, Genetics, and Evolution* 48 (2017): 10-18. DOI: 10.1016/j.meegid.2016.11.029

ONE HEALTH WORKFORCE:
THE NEXT GENERATION

> "From PREDICT I have gained knowledge that the wildlife can be a reservoir for a lot of zoonotic viruses and have gained a lot of experiences on methods such as how to collect specimens from bats and rodents in the field and also testing for viruses in the laboratory. PREDICT has been valuable in Cambodia for the good communication and collaboration between IPC and NAHPRI, with IPC training our staff in PREDICT laboratory techniques. The project has identified some viruses in domestic animals and wildlife for the first time in Cambodia and has provided great awareness for early prevention of disease between animal and human. Finally, PREDICT gave a lot of advances for scientists here in the One Health approach."

–Dr. Ren Theary,
Veterinarian
Cambodia's National
Animal Health &
Production Institute

PREDICT
CHINA

ONE HEALTH IN ACTION (2009-2019)

Ten-year joint effort of multi-sectoral partners to strengthen the emerging infectious disease surveillance system and disease response and preparedness capacity in China

In the highly biodiverse southern region of China, interactions among humans, wildlife, and livestock are increasing as a result of agricultural intensification, population movements, and urbanization. These changes provide a favorable context for the emergence of zoonotic disease, posing a public health threat particularly to the rural communities where frequent contact with animals occurs, but disease prevention measures are inadequate.

PREDICT China has worked with scientists, communities, and policy makers to discover zoonotic viruses among animal populations before emerging and causing potential pandemics. The project has allowed for an improved understanding of the pathogenesis of zoonotic viruses and the risk of emergence among human communities with the goal of developing targeted approaches to preventing and responding to zoonotic diseases.

During the past 10 years, the PREDICT project increased capacity of a One Health approach for emerging infectious disease prevention and response. The PREDICT team conducted concurrent virus surveillance among wild animals and human populations in high-risk regions in China, focusing on southern China, which is considered a “hotspot” for emerging zoonotic diseases in areas undergoing continuous land use change and overexploitation of natural resources. The PREDICT China team also collected human behavioral data to understand the risk factors for zoonotic disease emergence and identify appropriate social and behavioral interventions to reduce risk.

The PREDICT team worked with local laboratory partners to carry out virus characterization and pathogenesis studies to assess the zoonotic potential of detected viruses from surveillance, and developed serological assays for select bat coronaviruses. With support from the PREDICT modeling team, PREDICT China was able to use data collected over the past 10 years to better understand virus evolution, host-pathogen dynamics, and general ecology of disease emergence.

LOCAL PARTNERS

- Chinese Center for Disease Control and Prevention
- Guangdong Provincial Center for Disease Control and Prevention
- Guangdong Provincial Institute of Public Health
- Institute of Microbiology, Chinese Academy of Sciences
- School of Health Sciences, Wuhan University
- Wuhan Institute of Virology, Chinese Academy of Sciences
- Yunnan Provincial Institute of Endemic Disease Control

80

DEVELOPED the One Health Workforce by training 80 people in China.

>7.3K

OPERATIONALIZED One Health surveillance and sampled over 7.3K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Wuhan Institute of Virology of Chinese Academy of Sciences
- Institute of Microbiology of Chinese Academy of Sciences

39,137
TESTS

69
PREDICT

39
KNOWN

DETECTED 108 unique viruses in both animal and human populations.

GUANGJIAN ZHU

Country Coordinator, *EcoHealth Alliance*

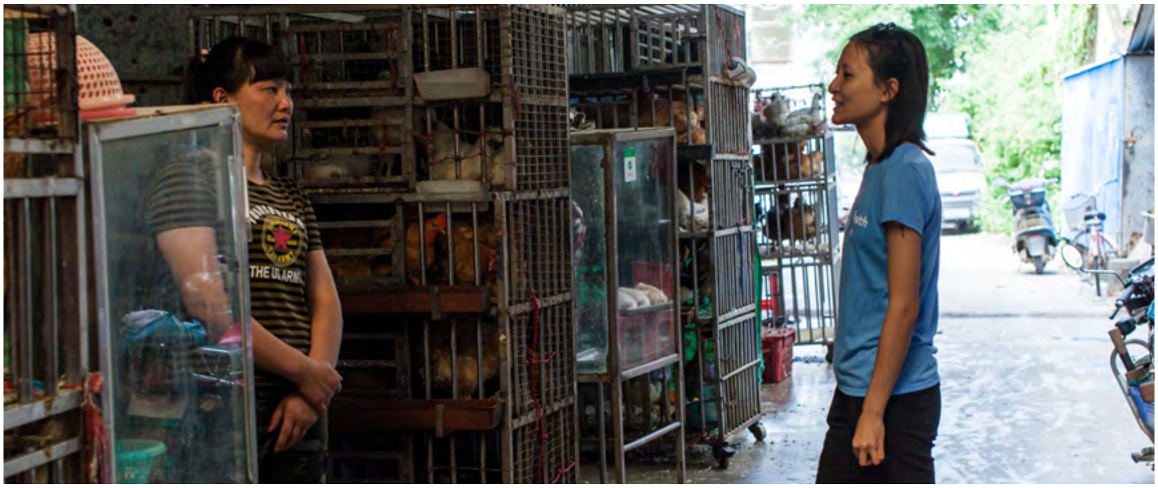
"During the past decade I worked for PREDICT, I have seen many scientists join the field for emerging infectious disease research. I hope my work through PREDICT can help set a gold standard for wildlife emerging infectious diseases field research practices in China."

ACHIEVEMENTS

PREDICT China supported other findings that bats are hosts to diverse coronaviruses (CoVs). The team also detected SARS-related CoVs, and adjacent studies showed some of them can infect human cells and cause disease in the lab mouse model for SARS. Adjacent studies also showed that some of the virus sequences we identified were used to demonstrate Remdesivir's broad efficacy against SARS-CoV and 'pre-pandemic' bat-CoVs. We also found serological evidence of SARS-related viruses in rural populations, suggesting that communities in rural China could be exposed to bat coronaviruses.

We detected a new CoV that may have bat origins, which caused a new disease and killed over 25,000 pigs in south China. This virus, SADS-CoV, is also able to infect human cells in the laboratory. Finally, adjacent work with collaborators identified the closest known relative to date, to the virus causing COVID-19 (SARS-CoV-2).

Throughout our work we have shared results and raised awareness with our Chinese Government partners and other partners including the US Embassy, USG agencies, the WHO, and the public on the knowledge gained of viruses in bats and the potential for spillover of viruses such as SAR-CoV-2. As the COVID pandemic unfolded, we continued to communicate widely on the value of the PREDICT project in understanding coronaviruses in bats, other animals and people.



ONE HEALTH SURVEILLANCE

To promote One Health collaboration in emerging zoonotic disease surveillance, PREDICT China worked with the China Centers for Disease Control and Prevention and its provincial departments, National Forestry and Grassland Administration, FAO China, agricultural universities, and hospitals/clinics to conduct virus surveillance in animal and human populations. Two sites were selected as the sampling sites for concurrent animal and human surveillance (Table 1). PREDICT China organized joint training on animal surveillance, conducted concurrent field sampling in animals and people, and established a communication and coordination network among multisectoral partners for timely results-sharing. PREDICT China's work has helped developed a culture of One Health collaboration in China that receives strong support from the government.

TABLE 1. Animals and humans sampled at concurrent community surveillance sites.

	NO. OF SAMPLING EVENTS	NO. OF ANIMALS SAMPLED	NO. OF HUMANS SAMPLED
CONCURRENT SITE 1	7	578	200
CONCURRENT SITE 2	8	473	300

VIRUS DETECTION

The PREDICT project's strategy for virus detection included screening samples using broadly reactive consensus PCR (cPCR) for priority virus groups, including coronaviruses, filoviruses, flaviviruses, paramyxoviruses, and influenza virus. Viruses detected via these assays were sequenced to investigate their relationship to known pathogens, and samples were prioritized for further characterization based on these results. This approach allows for detection of both known and novel viruses and improves our understanding of the potential for the virus to cause disease in humans and/or animals.

This approach led to the discovery of a remarkable diversity of viruses in bats, particularly coronaviruses, including close relatives of SARS-CoV and SARS-CoV-2. Our work provides further evidence that both of these human pathogens originated in bats, and that there is substantial potential for further spillover. Working in collaboration with NIAID-funded partners, we demonstrated that some of the newly discovered bat-CoVs were able to bind to human cells, infect them in vitro, and cause SARS-like disease in a lab animal model. Our findings led to the discovery that Remdesivir – the only drug currently known to have efficacy against COVID – could also disrupt replication of bat-CoVs that are on the cusp of emergence.

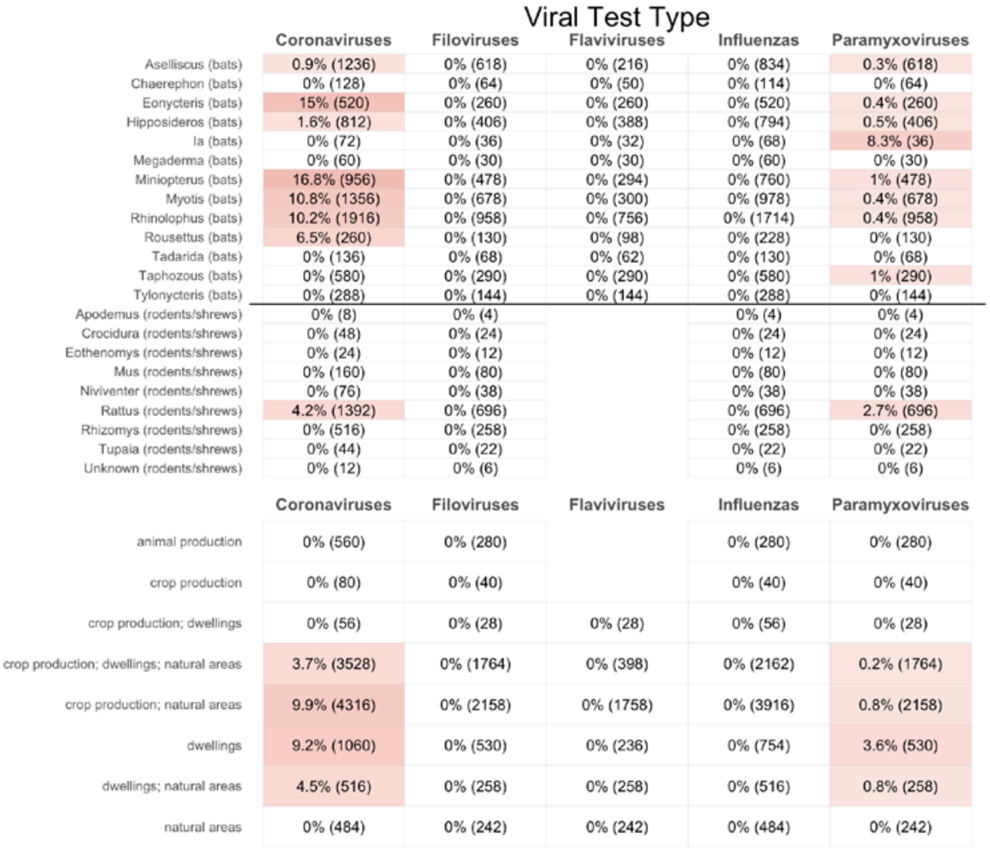
PREDICT China also discovered a range of paramyxoviruses in bats and both paramyxoviruses and CoVs in rodents. The team sampled wild and captive bred bamboo rats (a widely farmed and consumed species) to assess whether wildlife farming amplified virus prevalence and therefore risk of spillover. We found no evidence that it did, but the sample size was small for wild rodents given their widespread hunting.

Of the 29 CoVs detected during PREDICT-2, 26 were found in bat hosts while two species of commensal rodent, the Norway rat and Oriential house rat, were found to host three known CoVs: murine CoV, rodent CoV, and Longquan Aa mouse CoV. Insectivorous bats, primarily members of the genera *Hipposideros*, *Rhinolophus*, *Miniopterus*, and *Myotis*, were host to 23 of the 26 (88.5%) bat-CoVs, seven of which are novel viruses detected as a result of PREDICT project surveillance. Members of the fruit bat family, Pteropodidae, hosted five CoVs, two of which are novel viruses. Furthermore, two CoVs were found in both insectivorous and fruit bat species, demonstrating the ability of these viruses to infect a diversity of bat hosts.

Paramyxovirus (PMV) diversity was also high – a total of 20 unique viruses (three known and 17 novel) were detected in bats and rodents throughout PREDICT-2. Similar to CoV findings, insectivorous bats were the dominant hosts (18 of 20 viruses). However, bat-PMV abundance was relatively low, as each virus was only detected 1-2 times in contrast to bat-CoVs, where we detected each virus an average of 16 times (range: 1-69). In addition to the insectivorous bat-PMV findings, one novel virus, PREDICT_PMV-123, was found in a single fruit bat (Dawn bat; *Eonycteris spelaea*), and multiple Norway rats tested positive for the known Beilong virus.

The only virus to be detected in humans was influenza A, a well-known pathogen of humans and animals, found in an adult male. This single detection was a result of syndromic surveillance efforts in Guangdong Province.

TABLE 2. Virus heatmap for bats and rodents/shrews and by disease transmission interface



Viral Test Type

	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
Aselliscus (bats)	0.9% (1236)	0% (618)	0% (216)	0% (834)	0.3% (618)
Chaerephon (bats)	0% (128)	0% (64)	0% (50)	0% (114)	0% (64)
Eonycteris (bats)	15% (520)	0% (260)	0% (260)	0% (520)	0.4% (260)
Hipposideros (bats)	1.6% (812)	0% (406)	0% (388)	0% (794)	0.5% (406)
Ia (bats)	0% (72)	0% (36)	0% (32)	0% (68)	8.3% (36)
Megaderma (bats)	0% (60)	0% (30)	0% (30)	0% (60)	0% (30)
Miniopterus (bats)	16.8% (956)	0% (478)	0% (294)	0% (760)	1% (478)
Myotis (bats)	10.8% (1356)	0% (678)	0% (300)	0% (978)	0.4% (678)
Rhinolophus (bats)	10.2% (1916)	0% (958)	0% (756)	0% (1714)	0.4% (958)
Rousettus (bats)	6.5% (260)	0% (130)	0% (98)	0% (228)	0% (130)
Tadarida (bats)	0% (136)	0% (68)	0% (62)	0% (130)	0% (68)
Taphozous (bats)	0% (580)	0% (290)	0% (290)	0% (580)	1% (290)
Tylonycteris (bats)	0% (288)	0% (144)	0% (144)	0% (288)	0% (144)
Apodemus (rodents/shrews)	0% (8)	0% (4)		0% (4)	0% (4)
Crocidura (rodents/shrews)	0% (48)	0% (24)		0% (24)	0% (24)
Eothenomys (rodents/shrews)	0% (24)	0% (12)		0% (12)	0% (12)
Mus (rodents/shrews)	0% (160)	0% (80)		0% (80)	0% (80)
Niviventer (rodents/shrews)	0% (76)	0% (38)		0% (38)	0% (38)
Rattus (rodents/shrews)	4.2% (1392)	0% (696)		0% (696)	2.7% (696)
Rhizomys (rodents/shrews)	0% (516)	0% (258)		0% (258)	0% (258)
Tupaia (rodents/shrews)	0% (44)	0% (22)		0% (22)	0% (22)
Unknown (rodents/shrews)	0% (12)	0% (6)		0% (6)	0% (6)

	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
animal production	0% (560)	0% (280)		0% (280)	0% (280)
crop production	0% (80)	0% (40)		0% (40)	0% (40)
crop production; dwellings	0% (56)	0% (28)	0% (28)	0% (56)	0% (28)
crop production; dwellings; natural areas	3.7% (3528)	0% (1764)	0% (398)	0% (2162)	0.2% (1764)
crop production; natural areas	9.9% (4316)	0% (2158)	0% (1758)	0% (3916)	0.8% (2158)
dwellings	9.2% (1060)	0% (530)	0% (236)	0% (754)	3.6% (530)
dwellings; natural areas	4.5% (516)	0% (258)	0% (258)	0% (516)	0.8% (258)
natural areas	0% (484)	0% (242)	0% (242)	0% (484)	0% (242)

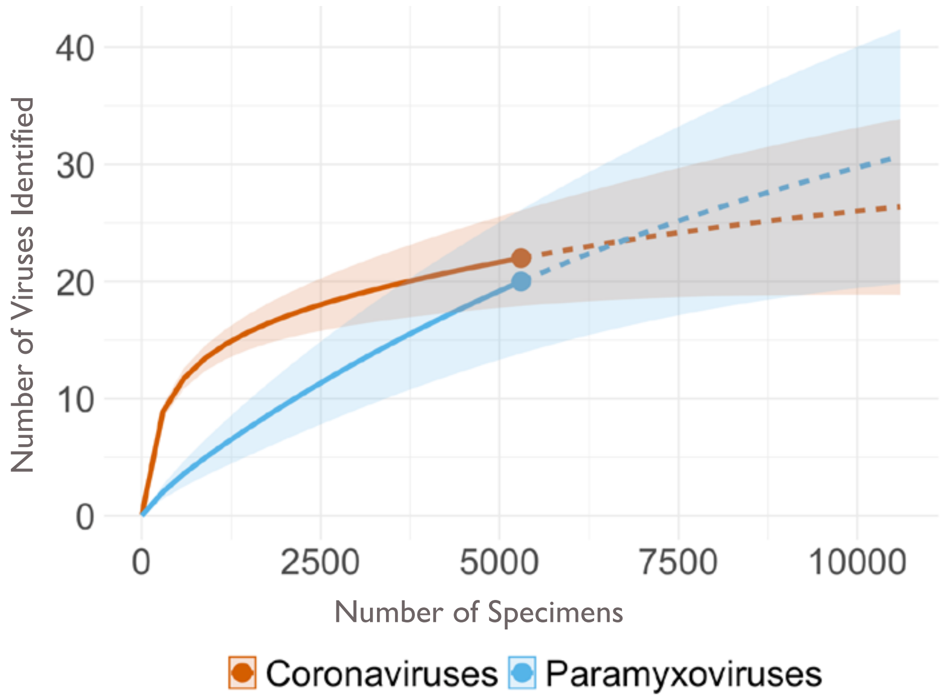


FIGURE 1. *Virus discovery in China. The extensive sampling of bats in China led to evidence of the beginning of saturation of the discovery curve for coronaviruses. This suggests that it would be possible to identify the majority of currently unknown CoVs in China, given relatively unsubstantial further sampling and testing.*

VIRUS TABLE

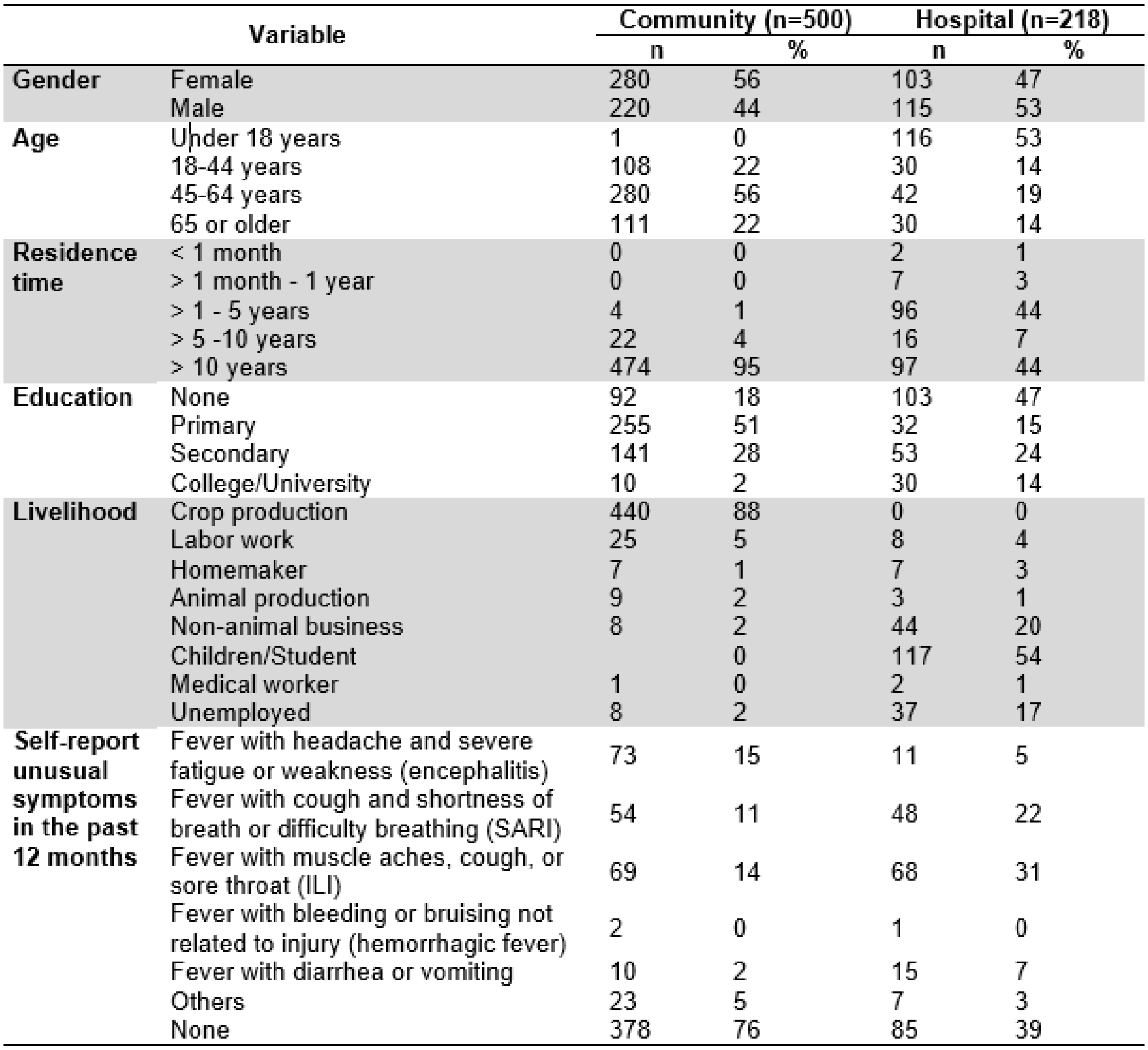
VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS
Coronavirus	PREDICT_CoV-22	Dawn Bat, Leschenault's Rousette	Xishuangbanna	43
	PREDICT_CoV-23	Dawn Bat	Xishuangbanna	1
	PREDICT_CoV-53	Intermediate Roundleaf Bat	Guilin	2
	PREDICT_CoV-60	Chinese Water Myotis	Chuxiong	17
	PREDICT_CoV-79	Least Horseshoe Bat	Guilin	37
	PREDICT_CoV-95	Intermediate Roundleaf Bat	Guilin	2
	PREDICT_CoV-107	Rickett's Big-Footed Bat	Chuxiong	3
	PREDICT_CoV-108	Himalayan Whiskered Myotis, Least Horseshoe Bat	Guilin	7
	PREDICT_CoV-111	Chinese Water Myotis	Chuxiong	1
	Bat coronavirus 1	Schreiber's Long-Fingered Bat, Small Long-Fingered Bat	Guangzhou, Jinning	69
	Bat coronavirus 512/2005	Rickett's Big-Footed Bat	Chuxiong	1
	Bat coronavirus Anlong 57/43	Himalayan Whiskered Myotis	Guilin	1

	Bat coronavirus HKU2	Chinese Horseshoe Bat, Himalayan Whiskered Myotis, Intermediate Horseshoe Bat, Least Horseshoe Bat, Stoliczka's Trident Bat, Thomas's Horseshoe Bat	Chuxiong, Guandong, Guilin, Jinning, Xishuangbanna	77
	Bat coronavirus HKU6	Chinese Water Myotis, Daubenton's Myotis, Himalayan Whiskered Myotis, Rickett's Big-Footed Bat, Small Long-Fingered Bat	Chuxiong, Guandong, Guilin, Jinning	62
	Bat coronavirus HKU8	Schreiber's Long-Fingered Bat	Guangzhou, Jinning	7
	Bat coronavirus HKU9	Dawn Bat, Intermediate Roundleaf Bat, Leschenault's Rousette	Chuxiong, Guilin, Xishuangbanna	11
	Bat coronavirus HKU10	Large-Eared Roundleaf Bat, Stoliczka's Trident Bat	Guilin, Xishuangbanna	6
	Bat coronavirus RS3376	Chinese Horseshoe Bat	Jinning	1
	Bat coronavirus RS4125/4259	Thomas's Horseshoe Bat	Xishuangbanna	1
	Hipposideros bat alphacoronavirus MJ/67C	Intermediate Horseshoe Bat, Stoliczka's Trident Bat	Xishuangbanna	6
	Myotis alphacoronavirus	Fringed Long-Footed Myotis	Chuxiong	1
	Rhinolophus/Hipposideros alphacoronavirus	Intermediate Horseshoe Bat	Xishuangbanna	4
	Rousettus bat coronavirus GCCDC1/346/356	Chinese Horseshoe Bat, Dawn Bat, Leschenault's Rousette	Jinning, Xishuangbanna	36
	Rousettus bat coronavirus/NRC-2	Leschenault's Rousette	Chuxiong	1
	SARS-related bat coronavirus RsSHC014	Intermediate Horseshoe Bat	Chuxiong	1
	SARS-related betacoronavirus Rp3/2004	Chinese Horseshoe Bat, Intermediate Horseshoe Bat, Thomas's Horseshoe Bat	Chuxiong, Guangzhou, Jinning, Xishuangbanna	17
	Longquan Aa mouse coronavirus	Norway Rat	Guangzhou	3
	Murine coronavirus	Norway Rat, Oriental House Rat	Guangzhou, Xishuangbanna	17
	Rodent coronavirus	Norway Rat	Guangzhou	14
Paramyxovirus	PREDICT_PMV-47	Great Evening Bat	Chuxiong	2
	PREDICT_PMV-49	Black-Bearded Tomb Bat, Unidentified Taphozous Bat	Guilin	2
	PREDICT_PMV-88	Intermediate Horseshoe Bat	Xishuangbanna	1
	PREDICT_PMV-89	Chinese Horseshoe Bat	Jinning	1
	PREDICT_PMV-90	Black-Bearded Tomb Bat	Guilin	1
	PREDICT_PMV-123	Dawn Bat	Xishuangbanna	1
	PREDICT_PMV-129	Chinese Horseshoe Bat	Guangzhou	1
	PREDICT_PMV-130	Fringed Long-Footed Myotis	Chuxiong	1
	PREDICT_PMV-134	Chinese Horseshoe Bat	Guilin	1
	PREDICT_PMV-135	Stoliczka's Trident Bat	Xishuangbanna	1
	PREDICT_PMV-136	Unidentified Taphozous Bat	Guilin	1
	PREDICT_PMV-157	Schreiber's Long-Fingered Bat	Jinning	2
	PREDICT_PMV-158	Schreiber's Long-Fingered Bat	Jinning	2
	PREDICT_PMV-162	Schreiber's Long-Fingered Bat	Jinning	1
	PREDICT_PMV-164	Stoliczka's Trident Bat	Xishuangbanna	1
	PREDICT_PMV-165	Chinese Water Myotis	Chuxiong	1
	PREDICT_PMV-166	Chinese Water Myotis	Jinning	1
	Bat paramyxovirus BtHp-ParaV/GD2012	Intermediate Roundleaf Bat	Guilin	2
	Bat paramyxovirus/B16-40	Schreiber's Long-Fingered Bat	Jinning	1
	Beilong virus	Norway Rat	Guangzhou	19
Influenza virus	Influenza A	Human	The 1st Affiliated Hospital of Shantou University	1
Total				**493**

EPIDEMIOLOGIC & BEHAVIORAL RISK

For PREDICT-2, our team in China conducted surveillance in both community and clinical settings by employing an integrated biological-behavioral surveillance approach. Through this approach, PREDICT China aimed to assess spillover potential of emerging zoonotic viruses at high-risk, animal-human interfaces and to use an evidence-based approach to identify behavioral risk factors associated with those interfaces. Ultimately this surveillance resulted in the development of risk-mitigation strategies tailored to local contexts, including community education around our *Living Safely with Bats* risk reduction and behavior change communication resource.

DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS



Variable		Community (n=500)		Hospital (n=218)	
		n	%	n	%
Gender	Female	280	56	103	47
	Male	220	44	115	53
Age	Under 18 years	1	0	116	53
	18-44 years	108	22	30	14
	45-64 years	280	56	42	19
	65 or older	111	22	30	14
Residence time	< 1 month	0	0	2	1
	> 1 month - 1 year	0	0	7	3
	> 1 - 5 years	4	1	96	44
	> 5 -10 years	22	4	16	7
	> 10 years	474	95	97	44
Education	None	92	18	103	47
	Primary	255	51	32	15
	Secondary	141	28	53	24
	College/University	10	2	30	14
Livelihood	Crop production	440	88	0	0
	Labor work	25	5	8	4
	Homemaker	7	1	7	3
	Animal production	9	2	3	1
	Non-animal business	8	2	44	20
	Children/Student		0	117	54
	Medical worker	1	0	2	1
	Unemployed	8	2	37	17
Self-report unusual symptoms in the past 12 months	Fever with headache and severe fatigue or weakness (encephalitis)	73	15	11	5
	Fever with cough and shortness of breath or difficulty breathing (SARI)	54	11	48	22
	Fever with muscle aches, cough, or sore throat (ILI)	69	14	68	31
	Fever with bleeding or bruising not related to injury (hemorrhagic fever)	2	0	1	0
	Fever with diarrhea or vomiting	10	2	15	7
	Others	23	5	7	3
	None	378	76	85	39

PREDICT China conducted biological-behavioral surveillance among rural residents in Yunnan, Guangxi, and Guangdong districts of Southern China, where the team had previously identified SARS-related CoVs in bats. In addition to the samples collected for virus detection, the PREDICT team collected serum samples from people that were evaluated for antibodies against four bat-borne coronaviruses (using a new ELISA assay based on selected nucleocapsid proteins). Surveys were administered to collect data on animal-human contact and zoonotic disease spillover risk. In this research, almost 20% of participants reported severe acute respiratory infections (SARI) and/or influenza-like illness (ILI) symptoms in the past year. Risk factors associated with these self-reported symptoms included poultry, carnivore, rodent/shrew, or bat contact along with socioeconomic factors, such as income and district of residence. Nine participants (0.6%) tested positive for antibodies against bat coronaviruses, suggesting bat coronavirus spillover in individuals in these communities. These results highlight the utility of an hypothesized early-warning system under non-outbreak conditions to detect the spillover event of emerging zoonotic diseases by extending the traditional clinical-based surveillance to at-risk communities.

The decade-long PREDICT project allowed the team to establish a biobank and database which will enable cost-effective future surveillance programs and in-depth studies for zoonotic disease prevention and control.

BEHAVIORAL RISK INVESTIGATIONS

PREDICT China also conducted ethnographic interviews and field studies in the rural communities of Yunnan, Guangxi, and Guangdong provinces. Data were analyzed to identify both risk and protective factors for zoonotic disease emergence at the individual, community, and policy levels (Figure 2). A total of 88 ethnographic interviews and 55 field observations were conducted at nine sites. The study provides evidence of frequent animal-human interactions in these communities and identifies key behavioral risk factors that can be targeted for mitigation strategies to reduce the risk of disease emergence. Existing local programs and policies around human and animal health, community development, and conservation are considered effective resources for developing cost-effective strategies to mitigate zoonotic disease risks.

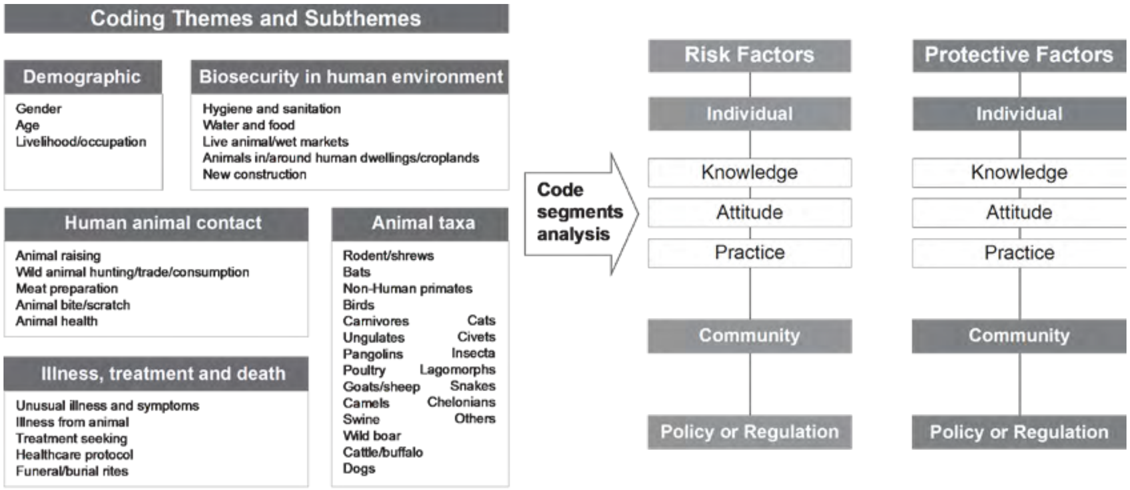


FIGURE 2. Qualitative method to identify the risk factors of zoonotic disease emergence.

Given the likely large number of undiscovered zoonotic pathogens currently in circulation in China, measures to reduce wildlife-human contact due to specific behaviors are urgently needed. This is particularly important for rural communities where close contact with bats and rodents was reported and zoonotic pathogens have been detected in animal populations. Enforcement of current wildlife protection policies coupled with capacity building in local clinics would likely significantly reduce high-risk contact between humans, wildlife, and livestock and risk for disease emergence and spread.

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

PREDICT China presented the results of surveillance actitivities to agency leads from the China CDC, Provincial and city CDCs in Yunnan, Guangdong, Beijing, and Shanghai. Behavioral risk surveys at all sites included presentation of results to communities where sampling and analysis took place.

CAPACITY STRENGTHENING

Although a national emerging infectious disease surveillance and reporting system was established in China following the SARS outbreak in 2002, the clinical-based effort is reaction-driven versus a proactive approach. PREDICT China worked with Chinese researchers and the government to expand surveillance to animals for early detection and proactive prevention. By working with both humans and animals, PREDICT China built communication and collaboration platforms which brought multisectoral stakeholders together for more effective and efficient surveillance.

In addition, the PREDCT China field team has significantly contributed to increased capacity for in-country field practices by providing training to different ecology, zoology, and virology research groups on field biosafety and humane animal sampling. Through the in-country partners, training was conducted for broad external partners from African and Asian countries.

OUTBREAK PREPAREDNESS & RESPONSE

A novel bat-origin coronavirus, swine acute diarrhea syndrome coronavirus (SADS-CoV), caused fatal swine disease outbreaks in Guangdong Province, during 2016-2018, leading to the death of more than 25,000 pigs. PREDICT China worked closely with agricultural researchers to identify the pathogen and investigate the ecology of transmission to provide recommendations for prevention measures. In the two years following the outbreak, PREDICT China has conducted regular surveillance among bat populations around farms in Guangdong Province, and worked with the global modeling team to assess the potential for future spillover from bats to pigs across China.

PRACTICAL IMPLICATIONS

- Identification of hundreds of bat-origin CoV sequences
- Discovery of a large diversity of CoVs closely related to SARS-CoV and SARS-CoV-2
- Identification of bat CoVs with clear potential to emerge directly in people
- Identification of a novel bat-origin virus, SADS-CoV, causing widespread outbreak in pigs in Southern China

FURTHER READING

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SPECIAL FEATURES

ONE HEALTH ACTION RESPONDING TO THE FATAL SWINE ACUTE DIARRHEA (SADS) SYNDROME OUTBREAKS

From October 2016, a fatal swine disease outbreak was observed in a pig farm in Guangdong province, China, causing severe acute diarrhea and vomiting, leading to death in newborn piglets (younger than five days) with a mortality of 90%. By May 2017, the disease had caused 24,693 piglet deaths at four farms. The clinical signs and preliminary lab testing suggested it was a novel disease caused by coronaviruses.

Learn more at **p2.predict.global/strengthening-health-security**

UNDERSTANDING THE CROSS-SPECIES TRANSMISSION OF BAT CORONAVIRUSES IN CHINA

Over the past decade, PREDICT China's surveillance efforts have generated a rich database with over 500 bat coronavirus (CoV) sequences. This includes alphacoronavirus sequences from 41 bat species and betacoronavirus sequences (the group that includes SARS-related CoVs) from 31 bat species. Our team has used these data to analyze the evolutionary origins and potential for cross-species transmission of bat CoVs in China.

Learn more at **p2.predict.global/insights**

ENHANCING THE CHINA-US COLLABORATION ON THE ECOLOGY & EVOLUTION OF EMERGING INFECTIOUS DISEASES (EID) RESEARCH

To counter the significant global health threat that emerging infectious diseases (EIDs) represent, China and the US have been leading efforts in preparedness with unparalleled resources, widespread engagement, and national and geopolitical imperatives to contribute to global health security. This commitment has been essential to the advancement of our understanding of pandemic threats. Even though the US and China have both invested in EID research, integrated scientific studies with strong ecological and evolutionary components are not yet well-supported.

Learn more at **p2.predict.global/insights**

PREDICT
INDIA

ONE HEALTH IN ACTION (2014-2020)

The Indian subcontinent is known for its extraordinary biodiversity. Due to its unique geographical location and evolutionary history, India contains multiple biodiversity hotspots – globally important regions with large number of unique species. Such hotspots are still being explored and are expected to be a treasure trove of unknown wildlife – from microorganisms to vertebrates. In addition to the hotspots, vast number of Indians still live close to natural habitats and regularly interact with wildlife – both directly and indirectly through their livestock. India is also a rapidly developing country. As a result, vast natural areas of the country are being modified. Such anthropogenic modifications may often create novel interfaces between humans and wildlife, which may eventually cause spillover of novel zoonotic pathogens.

Pathogens shared between people and animals result in millions of deaths annually and a single outbreak may cause economic losses amounting to trillions of dollars. Such tremendous losses are incurred because response to virus outbreaks are often late. These diseases represent major public health risks especially to low- and middle-income countries. Therefore, early detection and prevention of zoonotic spillover and spread are key to reducing the impact of epidemics and pandemics.

Given India's high population density and increasing regional and international connectivity, local outbreaks are likely to rapidly cross national boundaries causing pandemics. Therefore, for the benefit of both Indian and the global populations, potential EIDs must be identified and their risks characterized in detail. In India, the PREDICT project focused efforts on the highest risk locations and interfaces, where animals and people share changing landscapes, and diseases of unknown origin continue to take a significant toll. An example being the state of Uttar Pradesh, which has among the highest rates of undiagnosed encephalitis in India. The project was led by the Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPIMS) in Lucknow in partnership with EcoHealth Alliance.

LOCAL PARTNERS

- Chief Medical Officer Gorakhpur, Government of Uttar Pradesh
- Community Leaders/representatives, Maharajganj Field area, Uttar Pradesh
- Forest and Wildlife Department, Government of Uttar Pradesh
- Health Department, Government of Uttar Pradesh
- Medical College, Gorakhpur, Government of Uttar Pradesh
- Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPIMS), Lucknow
- USAID India

20

DEVELOPED the One Health Workforce by training 20 people in India.

84

OPERATIONALIZED One Health surveillance and sampled 84 animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Sanjay Ghandi Postgraduate Institute of Medical Sciences

1,127
TESTS

6
KNOWN

DETECTED 6 unique viruses in human populations.

RAJESH BHATIA

Former Director Communicable Diseases, *WHO Regional Office for South-East Asia, New Delhi, India*

"PREDICT enhanced national capacity and strengthened global security against emerging threats of novel zoonotic viruses. Augmented advocacy on One Health approach is likely to catalyze the policy decisions to accelerate OH implementation across several priority health issues. The project should be scaled up and integrated into the national disease surveillance programme."

HARJEET SINGH MAAN

Laboratory Lead, *Sanjay Gandhi Postgraduate Institute of Medical Science*

"I have since taken my expertise in microbiology to work as a Senior Scientist at the State Virology laboratory, at Gandhi Medical College in Bhopal Madhya Pradesh, India, where I am now in charge of a virology lab."

ACHIEVEMENTS

- Identified high-risk areas and performed site characterization within rural/agrarian Maharajganj district, Uttar Pradesh; subsequently conducted wildlife surveillance and biological sampling of bats, rodents, and non-human primates to discover emerging zoonotic disease threats
- Engaged in community partnership building and syndromic surveillance at a rural Maharajganj district clinic and conducted surveillance and behavioral risk investigations with patients
- Trained the laboratory team at SGPIMS in disease detection, outbreak response, biosafety and PPE, cold chain management, laboratory safety, and ethical research practices, all skills related to the detection of priority zoonotic diseases and emerging viral threats; as a result, the lab now has adequate capacity to safely detect known and novel viral threats and to test for virus groups that include priority zoonotic diseases
- Detected 6 known viruses in samples collected from febrile patients at our One Health surveillance site including influenza A, the known human coronavirus 229E, 3 known human parainfluenzaviruses, and West Nile virus
- Regularly participated in GHSA India meetings to present PREDICT project surveillance activities

ONE HEALTH SURVEILLANCE

In India, the PREDICT project conducted One Health surveillance in the Maharajganj district, Uttar Pradesh and its vicinity. Maharajganj is a border region between India and Nepal and as such is a potential corridor of transboundary transmission of emerging infectious diseases. The mostly rural, agrarian site is home to rodents, rhesus macaques, and bats that have frequent contact with people. Its proximity to the Nepal border also results in transboundary livestock movement. The Maharajganj district has a history of severe outbreaks of Acute Encephalitis Syndrome, AES, a clinical condition with symptoms that include high fever, confusion, disorientation, headache, and in severe cases, coma. These symptoms may result from multiple known pathogens, including Japanese encephalitis virus, Herpes simplex virus, enteroviruses, Chandipura virus, and Nipah virus. But, these symptoms can also be caused by as of yet unknown pathogens which, like Nipah, may spillover into people from wildlife populations. Our PREDICT team in India put One Health in action to sample wildlife and people in partnership with a local hospital, a hotspot for AES and febrile illnesses.

VIRUS DETECTION

The PREDICT project's strategy for virus detection included screening samples using broadly reactive consensus PCR (cPCR) for priority virus groups, including coronaviruses, filoviruses, flaviviruses, and paramyxoviruses as well as influenza virus. Positives detected using these assays were sequenced to identify the viruses and compare their relationship to known pathogens, and viruses were prioritized for further characterization. This approach allows for detection of both known and novel viruses and improves our understanding of the presence and diversity of viruses, as well as potential pathogens, in humans and animals.

In India, PREDICT project protocols were implemented at the laboratory of the Sanjay Gandhi Postgraduate Institute of Medical Science. The PREDICT program strengthens national laboratory systems by enabling disease detection through a One Health laboratory network based at partner labs mainly at the SGPIMS, Lucknow Uttar Pradesh. This lab maintains strong ties to the national system, and protocols and information will be shared openly with animal and human health labs working to actively improve interlinkages. Through in-service trainings, the PREDICT project provided opportunities for staff from the national system to enhance skills in virology, quality systems, biosafety, lab safety, and methods for detecting zoonotic diseases and emerging threats.

VIRUS FINDINGS IN PEOPLE

A total of 129 samples were tested from 65 people enrolled at the local Maharajganj district clinic and six known viruses from four virus groups were detected.

A strain of human coronavirus 229E was detected in six people with febrile illness sampled at the clinic. This is a strain of the known alphacoronavirus human coronavirus 229E. This human 229E virus is known to cause respiratory illness in people.

Influenza A was detected in 22 people (13 children and nine adults) with fever and influenza-like illness sampled at the clinic. This is the known virus Influenza A; influenza viruses are important pathogens in animals and people; therefore subtyping was recommended.

Three known paramyxoviruses were also detected. The known human parainfluenzavirus 2 was detected in three people (two children and one adult) with febrile illness sampled in the clinic. A strain of the known human parainfluenzavirus 1 was also detected in 11 people (seven children and four adults) with febrile illness sampled in a clinic. Finally, a strain of human parainfluenzavirus 3 was detected in 6 people (three children and three adults) with febrile illness sampled in the clinic. Human parainfluenzaviruses are major causes of lower respiratory tract infections in infants and elderly persons. Human parainfluenzavirus 3 virus has been previously found in other non-human primates but further studies are needed to determine whether it is transmitted between humans and non-human primates. PREDICT project investigators continue investigations to further characterize this virus.

We also detected a strain of West Nile virus (a known member of the flavivirus family) in three people (one adult and two children) sampled at the clinic. West Nile virus is spread by mosquitoes and causes few or no symptoms in the majority of people. About 20% of people develop a fever, headache, vomiting, or a rash and in less than 1% encephalitis or meningitis occurs.

Finally, several individuals (n=11) exhibited co-infections with the following combinations of viruses:

- Influenza A and a strain of human parainfluenzavirus 1 (n=3)
- Influenza A and a strain of human parainfluenzavirus 2 (n=1)
- Influenza A and a strain of human parainfluenzavirus 3 (n=2)
- Influenza A, a strain of human parainfluenzavirus 1, and a strain of human parainfluenzavirus 3 (n=1)
- Influenza A, a strain of human parainfluenzavirus 1, and a strain of human coronavirus 229E (n=2)
- Influenza A, a strain of human parainfluenzavirus 1, and a strain of West Nile virus (n=1)
- A strain of Human Parainfluenzavirus 1 and a strain of West Nile virus (n=1)

Samples from a total of 19 animals (seven bats, four non-human primates, and eight rodents/shrews) were also tested for the five virus groups. There were no viruses detected in these samples.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	Coronavirus 229E (Human strain)	Human	Clinic (Maharajganj district)	6	0	6
Paramyxovirus	Human parainfluenzavirus 1	Human	Clinic (Maharajganj district)	11	0	11
	Human parainfluenzavirus 2	Human	Clinic (Maharajganj district)	3	0	3
	Human parainfluenzavirus 3	Human	Clinic (Maharajganj district)	6	0	6
Influenza virus	Influenza A	Human	Clinic (Maharajganj district)	22	0	22
Flavivirus	West Nile virus	Human	Clinic (Maharajganj district)	3	0	3
Total				**36***	**0**	**36***

*Numbers do not total due to individuals having co-infections with multiple viruses

CAPACITY STRENGTHENING

In India, the PREDICT project lead implementing partner is SGPIMS, Lucknow, a tertiary care hospital and a premier research institute in medical sciences. Though the project, SGPIMS provided opportunities for students, interns, and staff to engage in One Health surveillance and enhance core One Health professional skills required for conducting field investigations (community sensitization and outreach on zoonotic disease risks, biosafety and PPE, safe sample collection from wildlife, livestock, and people, cold chain, safe sample storage and transport, behavioral risk investigations, and data management). The PREDICT project also provided multiple in-person trainings on safe and effective surveillance and sample collection to health professionals and health care workers in Gorakhpur and Maharajgan, empowering India's front line health workforce.

In addition, the PREDICT project provided technical expertise to the SGPIMS lab team in all core skills required to detect priority zoonotic diseases and emerging viral threats. This is a major capacity gain for an apex laboratory in Lucknow, a state which has among the highest rates of unknown acute encephalitis cases in India.

Finally, to encourage multi-sectoral information sharing across animal and human health sectors, the PREDICT team distributed reports on laboratory, capacity, and surveillance progress to the USAID Mission, the Global Health Security Agenda (GHSA) cell (including partners with the US Center for Disease Control in New Delhi), and local institutions such as the Forestry Department, government partners, and university collaborators. In an effort to coordinate across local institutions, the PREDICT team also shared information and reported to the Indian Council of Medical Research, National Centre for Disease Control, and UP State Health Department. In addition to multi-sectoral collaboration, cross-border collaboration was also implemented, as the PREDICT India field lead trained with the PREDICT Bangladesh team in Bangladesh, an illustration of the importance of the PREDICT network and the value of South-South collaboration.

PRACTICAL IMPLICATIONS

STRENGTHENED STATE LABORATORY CAPACITY FOR DETECTION OF EMERGING VIRAL THREATS

The PREDICT project in India empowered and established a One Health laboratory team, with the capability to safely test samples from both wildlife and human populations. This is the first lab in Lucknow, and one of the first in India within the GHSA lab network, that has the capacity to screen both human and wildlife samples for priority zoonotic diseases and newly emerging threats such as Disease X.

PUT ONE HEALTH IN ACTION IN NORTHERN INDIA

The PREDICT project's work in northern India shed light on the causes and risks for undiagnosed acute encephalitis and helped develop capacity for surveillance and disease detection, critical skills for empowering the health workforce and for sustaining effective disease investigations and response to disease threats.

PREDICT
INDONESIA

ONE HEALTH IN ACTION (2009-2020)

INDONESIA

Supporting the Government of Indonesia to strengthen health security and prevent, detect, and rapidly respond to the spillover of infectious pathogens from wildlife to humans

Indonesia has abundant biodiversity, including non-human primates, rodents, and bats. High rates of tropical deforestation, thriving wildlife trade and hunting networks, and a growing human population threaten Indonesia's unique biodiversity and increase the risk for zoonotic disease emergence. As an archipelago, Indonesia's seas and oceans may have historically limited the distribution of wild animal species and the pathogens they may carry. However, globalization and resulting changes in human activities are increasing movement across and beyond the country. This movement increases the risk for wildlife-human contact and the potential for cross-species transmission and spread of viruses. Rapid ecological change, such as clearing of peat swamps and tropical rain forests for palm oil plantations, are bringing humans into closer contact with wildlife species than in the past.

Since 2009, the PREDICT project has been active in Indonesia and worked with the central government and local partners from the Ministry of Agriculture (Local Animal Health Offices at the Provincial and District levels) to better understand the mechanisms of viral spillover from wildlife to livestock and people and to strengthen capabilities for the detection of priority zoonotic diseases and other viruses at high-risk, animal-human interfaces. Through analysis of project data and findings, the PREDICT team was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats. PREDICT project surveillance was performed at several sites on the Island of Sulawesi, the fourth largest island in Indonesia. Sulawesi is an important region for wildlife trade and is undergoing rapid land conversion and exploitation of local fauna. PREDICT One Health surveillance sites were targeted to capture high-risk animal-human interfaces along the wildlife value chain in Sulawesi.

LOCAL PARTNERS

- Food & Agriculture Organization of the United Nations (FAO)
- Gorontalo University
- Indonesia One Health University Network
- International Federation of Red Cross and Red Crescent Societies
- Ministry of Agricultural Republic Indonesia – Directorate General of Livestock and Animal Health Services
- Ministry of Environment and Forestry Republic Indonesia
- Ministry of Health Republic Indonesia
- Ministry of Research, Technology, and Higher Education
- Noongan Hospital and a local Minihasa Regency clinic, North Sulawesi
- Sam Ratulangi University of Manado
- USAID Preparedness and Response
- World Health Organization of the United Nations (WHO)

Global Health Security Agenda

>250

DEVELOPED the One Health Workforce by training more than 250 people in Indonesia.

>3.6K

OPERATIONALIZED One Health surveillance and sampled over 3.6K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Primate Research Center of the Institut Pertanian Bogor (IPB University)
- Eijkman Institute for Molecular Biology (EIMB)

>49,395

TESTS

30

PREDICT

20

KNOWN

DETECTED 50 unique viruses in both animal and human populations.

TINA KUSUMANINGRUM

Field Coordinator, *Emerging Virus Research Unit, Eijkman Institute for Molecular Biology*

"There is no doubt that joining PREDICT is one of the best decisions in my researcher career. Learning how to design and implement a surveillance project, maintain networking and professional relationships with partners, and communicate the results back to the communities and decision makers – these were all exceptional experiences that will be very useful for my future career."

RACHMITASARI NOVIANA

Wildlife Data Manager, *Microbiology & Immunology Laboratory, PRC - IPB University*

"Since working with PREDICT Indonesia, I've learned so much and I have changed my point of view about animal and virus findings. I have also improved my ability in information technology. I am honored to be a member of the Data Management team."

ACHIEVEMENTS

- Trained >130 people in Indonesia's One Health workforce in core skills required for zoonotic disease surveillance and detection
- Sampled >3,600 individuals (2,966 animals and 673 people) at high risk interfaces with potential for exposure to and spillover of zoonotic viruses
- Interviewed >300 people to better understand the social and behavioral factors associated with exposure to zoonotic diseases
- Strengthened two laboratories essential for supporting the national laboratory system to improve the detection of priority zoonoses and other viruses, and improving capabilities for bioinformatics analysis
- With FAO and the Ministry of Agriculture, trained and empowered eight National Veterinary Disease Investigation Centers (DICs – Banjarbaru, Bukittinggi, Denpasar, Lampung, Maros, Medan, Subang, and Wates) to detect new and emerging viruses in domestic animals
- Provided government partners with disease detection support during two outbreak response investigations for diseases of unknown origin
- Promoted safe behaviors and practices for risk reduction in communities at-risk for exposure to zoonotic diseases
- Engaged with Government of Indonesia partners and advocated for zoonotic disease and prevention programs

ONE HEALTH SURVEILLANCE

The PREDICT project in Indonesia put One Health in action by sampling wildlife taxa and people at several sites on the Island of Sulawesi. Domestic animal surveillance was also performed at these same sites in collaboration with the Ministry of Agriculture and FAO-ECTAD. Sulawesi is an important site for emerging zoonotic diseases given the widespread and high-volume of wildlife trade, rapid land conversion, and increasing connectivity of communities via road building and other infrastructure changes. PREDICT project surveillance sites targeted high-risk animal-human interfaces along the wildlife value chain pathway in Sulawesi (primarily sampling bats and rodents, as well as non-human primates and other taxa). These sites include different stages of the wildlife value chain, from wild source populations of animals, to hunters and middlemen, to urban wildlife markets. Hunted wild animals, mostly bats and rodents, were sampled at hunting sites in Gorontalo, West Sulawesi, Southeast Sulawesi, and North Sulawesi provinces. Wild animals were also sampled in markets in North Sulawesi where the markets selling wildlife products are located.

Community-based surveillance and behavioral risk investigations occurred concurrently with wildlife sampling with the aim to detect virus sharing among people and animals and to characterize behavioral risk factors for exposure in Gorontalo, West Sulawesi, Southeast Sulawesi, and North Sulawesi provinces. Wildlife hunters, market vendors and wildlife consumers were enrolled as participants, and a wider sample of community members and occupations were also recruited. Clinical surveillance was performed at Noongan Hospital (Rumah Sakit Umum Daerah Noongan) and at a local clinic, both within Minahasa Regency, North Sulawesi. Sampling sites were chosen to capture individuals associated with the wildlife value chain that may be at risk for illness. Patients with acute febrile and respiratory illness were enrolled at clinics.

The PREDICT team coordinated with local hospitals, primary health care centers, community leaders, as well as universities to implement human behavioral surveillance and to conduct syndromic and community surveillance. This coordinated approach included several scoping visits to sensitize target communities in Sulawesi, training events with hospital staff and other personnel, and other meetings for partner engagement.

In Indonesia, human health is under the jurisdiction of the Ministry of Health and animal health is under split authority, as wildlife management is under the Ministry of Environment and Forestry, and animal health and husbandry under the Ministry of Agriculture. This shared governance of animal health creates an opportunity for miscommunication and unaligned agendas.

In both human and veterinary health sectors, government labs focus their surveillance on known diseases in humans and animals. As the PREDICT project's focus was to improve capacity to also detect novel viruses, we worked to fill in the gaps in data related to new and emerging viruses both in human and animal health.

One Health surveillance unified multi-sectoral stakeholders in Indonesia. Activities were initiated with permission requests from all relevant authorities, resulting in increased awareness and collaboration across the previously divided animal and human health sectors.

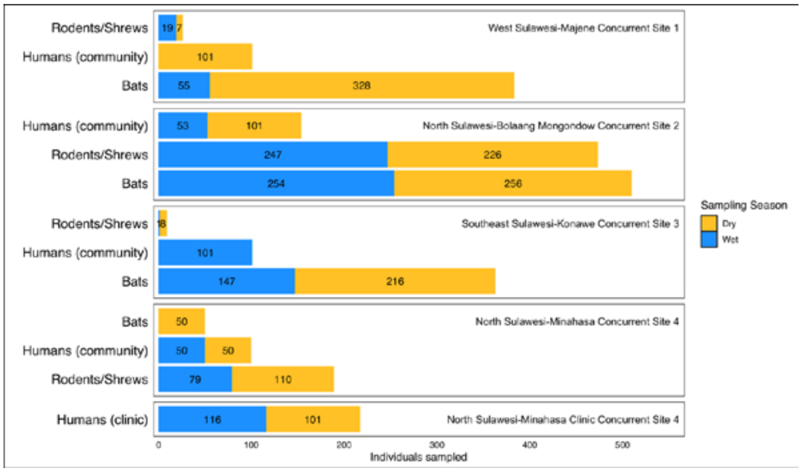


FIGURE 1. Summary of One Health sampling effort at multiple PREDICT sites in Indonesia. Gorontalo, an independent bat sampling site, is not represented here.

VIRUS DETECTION

VIRUS FINDINGS IN ANIMALS

The PREDICT project's strategy for virus detection included screening samples using broadly reactive consensus PCR (cPCR) for priority virus groups including coronaviruses, filoviruses, influenza viruses, flaviviruses, and paramyxoviruses. Positives detected using these assays were sequenced to identify the viruses and compare their relationship to known pathogens, and viruses were prioritized for further characterization. This approach allows for detection of both known and novel viruses and improves our understanding of the presence and diversity of viruses, as well as potential pathogens, in humans and animals.

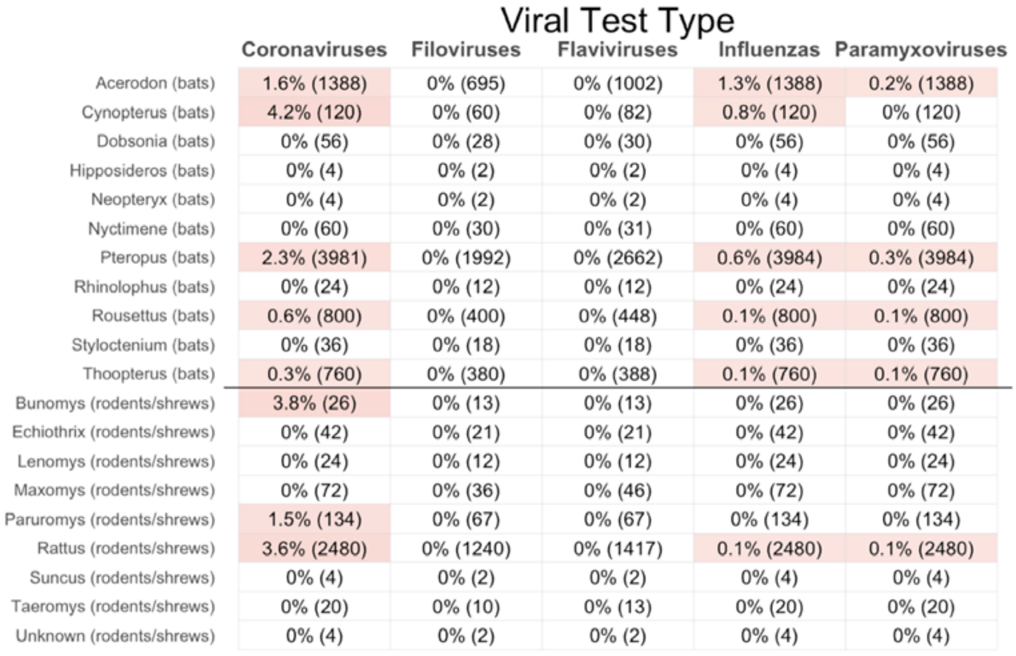
Using this approach, the wildlife laboratory safely tested 1,822 bat and 723 rodent oral and rectal swab samples. Of 3,618 bat specimens tested, 119 were positive for coronaviruses, 17 for paramyxoviruses, and 46 for influenza A. Of 1,393 rodent specimens tested, 57 were positive for coronaviruses, three for paramyxoviruses, and two for influenza A (Table 1). We also performed species identification by barcoding for 168 virus-positive bats, 71 virus-negative bats of various species, and all 723 rodent samples.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Clinic (Minahasa)	1	1	0
	PREDICT_CoV-16	Black Flying Fox	Gorontalo, Konawe, Majene	16	4	12
	PREDICT_CoV-67	Black Flying Fox, Spectacled Flying Fox	Gorontalo, Konawe, Majene, Parigi Mouton	48	9	39
	PREDICT_CoV-68	Black Flying Fox	Gorontalo, Majene	22	0	22
	PREDICT_CoV-73	Black Flying Fox, Sulawesi Fruit Bat	Bolaang Mongondow, Gorontalo, Konawe, Majene	19	10	9
	PREDICT_CoV-83	Black Flying Fox, Sulawesi Fruit Bat	Gorontalo	3	2	1
	Bat coronavirus HKU9	Black Flying Fox, Geoffroy's Rousette, Sulawesi Fruit Bat	Bolaang Mongondow, Majene	7	7	0
	Philippines/Diliman 1525G2/2008	Unidentified Cynopterus Bat, Unidentified Thoopterus Bat	Bolaang Mongondow	4	3	1
	Murine coronavirus	Black Rat, Giant Sulawesi Rat, Hoffmann's Sulawesi Rat, Montane Bunomys, Opossum Rat, Oriental House Rat, Ricefield Rat, Unidentified Rattus Rat, Yellow-Tailed Rat	Bolaang Mongondow, Gorontalo, Konawe, Majene, Minahasa	52	24	28
Paramyxovirus	Measles virus	Human	Minahasa	1	1	0
	Human parainfluenzavirus 4	Human	Clinic (Minahasa)	2	0	2
	PREDICT_PMV-10	Black Flying Fox	Gorontalo	1	0	1
	PREDICT_PMV-93	Sulawesi Fruit Bat	Gorontalo	1	1	0
	PREDICT_PMV-94	Black Flying Fox	Gorontalo	1	0	1
	PREDICT_PMV-95	Sulawesi Fruit Bat	Gorontalo	1	0	1
	PREDICT_PMV-100	Black Flying Fox	Gorontalo	1	0	1
	PREDICT_PMV-102	Black Flying Fox	Gorontalo	1	1	0
	PREDICT_PMV-111	Sulawesi Fruit Bat	Gorontalo	1	0	1
	PREDICT_PMV-112	Geoffroy's Rousette	Bolaang Mongondow	1	1	0

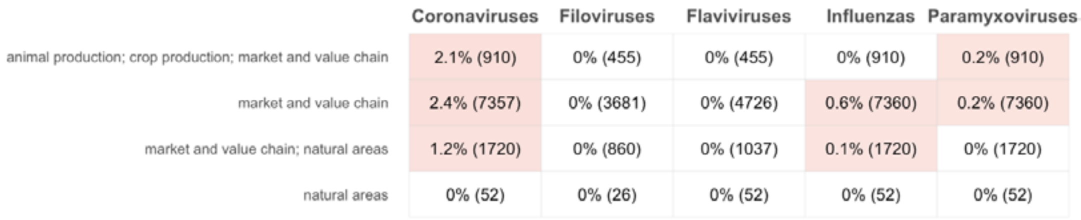
	PREDICT_PMV-121	Black Flying Fox	Majene	2	0	2
	PREDICT_PMV-122	Swift Fruit Bat	Bolaang Mongondow	1	0	1
	PREDICT_PMV-124	Black Flying Fox	Majene	4	0	4
	PREDICT_PMV-126	Black Flying Fox	Majene	1	0	1
	Pteropus poliocephalus paramyxovirus	Black Flying Fox	Gorontalo	1	0	1
	PREDICT_PMV-96	Unidentified Rattus Rat	Gorontalo	1	1	0
	PREDICT_PMV-154	Hoffmann's Sulawesi Rat	Minahasa	2	0	2
Influenza virus	Influenza A	Human, Black Flying Fox, Geoffroy's Rousette, Sulawesi Fruit Bat, Unidentified Cynopterus Bat, Unidentified Fruit Bat, Opossum Rat	Bolaang Mongondow, Gorontalo, Konawe, Noongan Hospital (Minahasa), Clinic (Minahasa)	66	34	32
	Influenza B	Human	Noongan Hospital (Minahasa), Clinic (Minahasa)	2	2	0
Flavivirus	Dengue virus serotype 2	Human	Noongan Hospital (Minahasa)	7	3	4
	Dengue virus serotype 3	Human	Noongan Hospital (Minahasa)	7	5	2
	Dengue virus serotype 4	Human	Noongan Hospital (Minahasa)	1	1	0
Picornavirus	Rhinovirus A	Human	Noongan Hospital (Minahasa), Clinic (Minahasa)	5	2	3
	Rhinovirus B	Human	Noongan Hospital (Minahasa)	1	1	0
	Rhinovirus C	Human	Clinic (Minahasa)	1	1	0
Total				**285**	**114**	**171**

Coronaviruses were the most frequently detected virus group and were detected in well-sampled wildlife genera including *Pteropus* spp. (flying fox fruit bats); *Cynopterus* spp. (short-nosed fruit bats); and *Rattus* spp. (rats) with an overall observed prevalence of between 2-4%. Influenza viruses and paramyxoviruses were found in some of these same groups of animals, but at a much lower proportion (<1% of samples were positive). The heatmap (Figure 2) summarizes the virus positives by each genus of wildlife sampled and tested from 2014-2019.



	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
Acerodon (bats)	1.6% (1388)	0% (695)	0% (1002)	1.3% (1388)	0.2% (1388)
Cynopterus (bats)	4.2% (120)	0% (60)	0% (82)	0.8% (120)	0% (120)
Dobsonia (bats)	0% (56)	0% (28)	0% (30)	0% (56)	0% (56)
Hipposideros (bats)	0% (4)	0% (2)	0% (2)	0% (4)	0% (4)
Neopteryx (bats)	0% (4)	0% (2)	0% (2)	0% (4)	0% (4)
Nyctimene (bats)	0% (60)	0% (30)	0% (31)	0% (60)	0% (60)
Pteropus (bats)	2.3% (3981)	0% (1992)	0% (2662)	0.6% (3984)	0.3% (3984)
Rhinolophus (bats)	0% (24)	0% (12)	0% (12)	0% (24)	0% (24)
Rousettus (bats)	0.6% (800)	0% (400)	0% (448)	0.1% (800)	0.1% (800)
Styloctenium (bats)	0% (36)	0% (18)	0% (18)	0% (36)	0% (36)
Thoopterus (bats)	0.3% (760)	0% (380)	0% (388)	0.1% (760)	0.1% (760)
Bunomys (rodents/shrews)	3.8% (26)	0% (13)	0% (13)	0% (26)	0% (26)
Echiothrix (rodents/shrews)	0% (42)	0% (21)	0% (21)	0% (42)	0% (42)
Lenomys (rodents/shrews)	0% (24)	0% (12)	0% (12)	0% (24)	0% (24)
Maxomys (rodents/shrews)	0% (72)	0% (36)	0% (46)	0% (72)	0% (72)
Paruromys (rodents/shrews)	1.5% (134)	0% (67)	0% (67)	0% (134)	0% (134)
Rattus (rodents/shrews)	3.6% (2480)	0% (1240)	0% (1417)	0.1% (2480)	0.1% (2480)
Suncus (rodents/shrews)	0% (4)	0% (2)	0% (2)	0% (4)	0% (4)
Taeromys (rodents/shrews)	0% (20)	0% (10)	0% (13)	0% (20)	0% (20)
Unknown (rodents/shrews)	0% (4)	0% (2)	0% (2)	0% (4)	0% (4)

We found a significant number of viruses detected in the market animal value chain, as compared to sites that were characterized as natural areas with free-ranging animals (Figure 3).



	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
animal production; crop production; market and value chain	2.1% (910)	0% (455)	0% (455)	0% (910)	0.2% (910)
market and value chain	2.4% (7357)	0% (3681)	0% (4726)	0.6% (7360)	0.2% (7360)
market and value chain; natural areas	1.2% (1720)	0% (860)	0% (1037)	0.1% (1720)	0% (1720)
natural areas	0% (52)	0% (26)	0% (52)	0% (52)	0% (52)

FIGURES 2 & 3. ***Heatmaps providing a summary of sampling effort and detection of positives for the five priority virus groups: coronaviruses, filoviruses, flaviviruses, influenza viruses, and paramyxoviruses. The data are summarized by host at the genus level, and broader taxonomic groups are shown in parentheses (Figure 2, top). Data are also summarized by interfaces at the sampling sites (Figure 3, bottom). The heatmap data show the percentage of virus positives that were confirmed by sequencing (%) and the number of PCR tests performed (in parentheses). The red color scales with increased virus positives. Host taxa or interfaces that did not test positive are shown in white.***

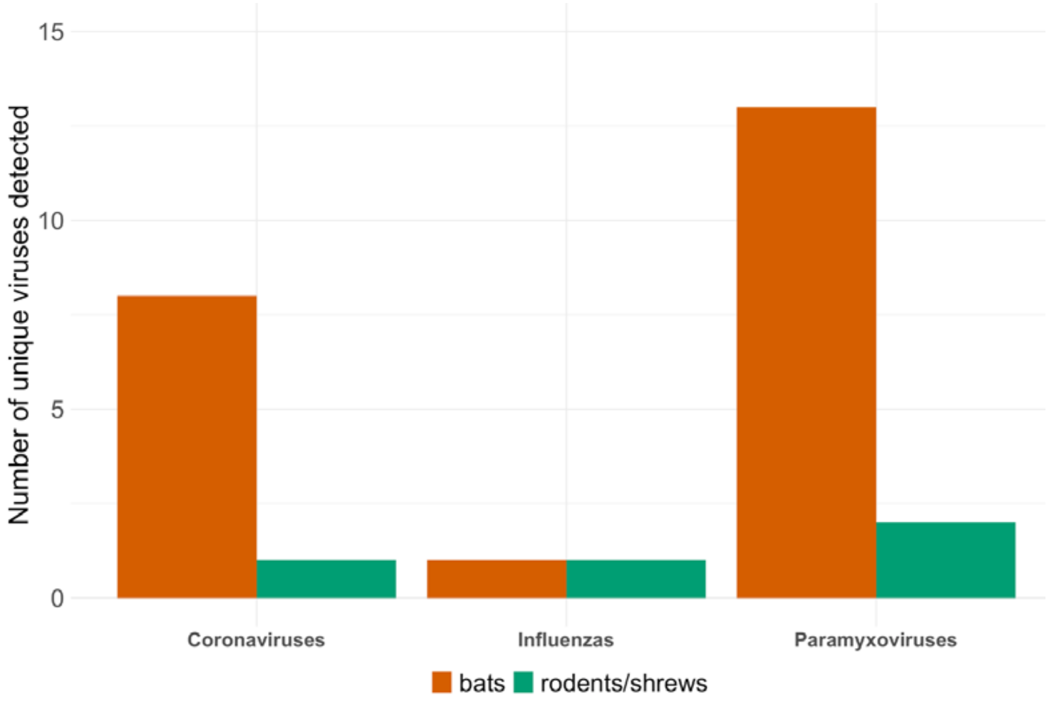


FIGURE 4. ***The number of unique viruses detected shown by taxa (bats in orange and rodents/shrews in green).***

Overall, a diversity of novel viruses were detected from known pathogen groups in humans and animals, including five new coronaviruses and 14 new paramyxoviruses. It is not known if these novel viruses identified pose a threat to human health, but they provide important baseline information on the diversity present and can help to develop a risk-mapping framework. Previously recognized viruses were also detected in wildlife, including influenza A and murine coronavirus. These findings for known viruses significantly expand the number of previously known mammal host species for these two virus species (see Figure 5) and improve our understanding of the natural host ranges for these viruses. Previous analyses (Olival et al., 2017; Johnson et al., 2015) have shown that the zoonotic, or spillover, potential of wildlife viruses is strongly tied to the breadth of hosts that a given virus is capable of infecting in nature.

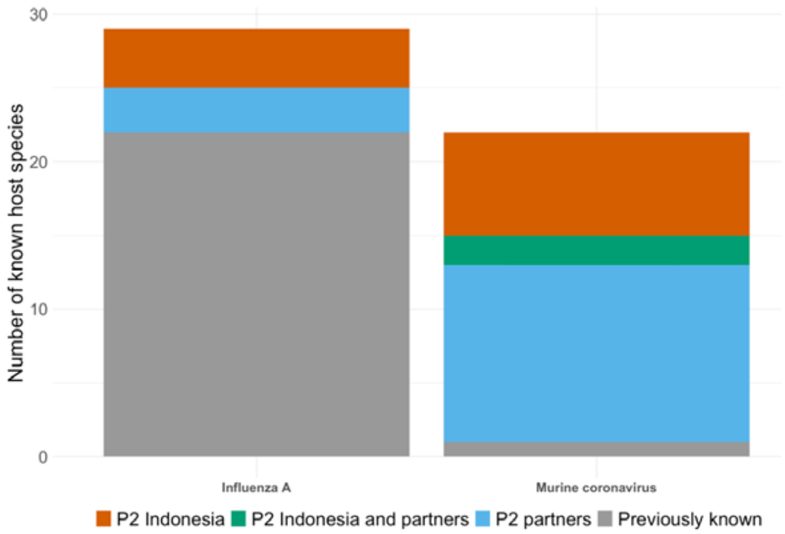


FIGURE 5. ***Expansion of the number of recognized mammal host species for previously-described viruses as a result of sampling in Indonesia. Each bar represents previously described (known) virus, i.e. those named by the International Committee on the Taxonomy of Viruses 8th edition. Previously known host species for each virus are based on Olival et al., 2017. The number of mammalian hosts for each virus are visualized using stacked bar plots, showing the number of pre-PREDICT-2 (P2; prior to 2015) recognized host species (gray portion), the number of newly recognized host species found in Indonesia (orange portion), and, where applicable, the number of newly recognized host species found in both Indonesia and other PREDICT-2 country sampling efforts (green portion) and the number of newly recognized host species found in other PREDICT-2 countries. Only previously described viruses detected in Indonesia in new host species are shown.***

VIRUS FINDINGS IN PEOPLE

The human laboratory safely collected biological specimens from 217 patients from Noongan Hospital and the clinic in North Sulawesi, as well as samples from 454 participants in the community. The team tested 1,352 specimens for paramyxoviruses, influenza viruses, coronaviruses, flaviviruses, and filoviruses using conventional consensus PCR methods. Two patients were positive for human parainfluenzavirus 4, 19 were positive for influenza A viruses, one for betacoronavirus 1 (OC43), 15 for dengue virus, and seven tested positive for rhinoviruses. Only one virus, measles virus, was detected in the community-based surveillance. These data show the effectiveness of using consensus virus group screening assays to detect important human pathogens not often included in surveillance efforts for both sick and healthy participants. Although influenza A was the only virus detected in both people and wildlife, we were able to collect important data on viruses circulating in both syndromic and community settings. The human viruses detected by site, specimen type, and frequency of detection are summarized in Table 2.

VIRUS NAME	VIRUS TEST TYPE	NUMBER OF DETECTIONS	SITE NAMES	SPECIMEN TYPES
INFLUENZA A	Influenzas	37	Noongan Hospital, Minahasa	Feces, nasopharyngeal swab, oral swab, oropharyngeal swab, nasal swab, blood (whole)
DENGUE VIRUS	Flaviviruses	15	Noongan Hospital	Blood (whole)
RHINOVIRUS A	Enteroviruses	5	Noongan Hospital, Minahasa	Oropharyngeal swb, nasal swab, nasopharyngeal swab
HUMAN PARAINFLUENZAVIRUS 4	Paramyxoviruses	2	Minahasa	Nasal swab
INFLUENZA B	Influenzas	2	Noongan Hospital, Minahasa	Oropharyngeal swab, oral swab
BETACORONAVIRUS 1 (OC43)	Coronaviruses	1	Minahasa clinic	Nasal swab
MEASLES VIRUS	Paramyxoviruses	1	Minahasa	Oropharyngeal swab
RHINOVIRUS B	Enteroviruses	1	Noongan Hospital	Oropharyngeal swab
RHINOVIRUS C	Enteroviruses	1	Minahasa	Nasal swab

TABLE 2. ***Virus findings in people by site, specimen type, and frequency of detection***

EPIDEMIOLOGICAL & BEHAVIORAL RISK

INSIGHTS FROM IN-DEPTH BEHAVIORAL RISK INVESTIGATIONS

In Indonesia a human questionnaire was administered in three provinces which each have different customs, cultures, and religious practices. The differences in each setting have implications for our findings related to the frequency of animal contacts and associated risk of exposure to zoonotic disease, as well as other differences such as hygiene practices, health-seeking behavior, and knowledge about zoonotic risk.

In general, the respondents at all study sites have implemented some hygiene practices in their household including for water treatment, food storage, and human waste management. Knowledge of the risk of zoonotic disease is still limited, even among individuals involved in the wildlife value chain, with only a few respondents under the perception that a wild animal can cause sickness. These preliminary findings imply that the communities, especially the ones categorized as high-risk, need to be better informed about zoonotic disease transmission.

We found that high-risk people such as wild meat hunters, vendors, and consumers lacked knowledge regarding the potential threat of zoonotic disease. It was found that these groups sometimes use traditional plants to treat wounds. While the active treatment of wounds shows the acknowledgment of risk from being bitten or scratched by an animal, the adoption of preventative measures, such as wearing personal protective equipment (PPE) while hunting, is not practiced.

Another interesting finding from the human survey was related to the knowledge of government regulation for wildlife protection. In 1999, the Government of Indonesia banned the hunting and sale of various wildlife species including large animals like babirusa and anoas. Some respondents said they knew about the regulation but continued to sell wildlife for economic reasons. This last finding presents a significant challenge to develop interventions for subsistence hunters despite existing knowledge of the government regulations. The development of solutions will require a multisectoral coordination by the government to find the most effective solution, as well as promotion of educational materials related to risk.

INSIGHTS FROM QUALITATIVE BEHAVIORAL RISK

Focusing on the thriving bushmeat trade in Sulawesi, the behavioral risk team conducted qualitative research to obtain a deeper understanding of how local communities interact with livestock, wildlife, bushmeat markets, and the surrounding bushmeat value chain. The multidisciplinary team, trained in qualitative research methodology and data collection used in-depth one-on-one interviews and focus group discussions to engage community members from 15 locations within the main sampling sites in North, West, and South Sulawesi.

The participants, many of whom were targeted for their involvement in the wildlife trade (hunters, bushmeat vendors, homemakers), described several types of contact with taxa such as rats, bats, wild boar, non-human primates, cuscus, snakes, anoas (wild cows), and yaki (non-human primates). Of these, rats, bats, and wild boar were the animals most often hunted, transported, and sold by the respondents. Among most vendors and hunters, it was more common to work with multiple taxa rather than exclusively with one, thus suggesting an increased risk for exposure to zoonotic viruses.

Among those questioned on injuries obtained while handling live or dead wildlife, rats appeared to be the source of most bites, and bats the source of most scratches. Health-seeking behaviors, particularly wound care following bites and scratches were inconsistent. While respondents generally had a history of accessing care at established clinical facilities, they mostly relied upon those services for more severe injuries. Many participants opted for traditional ethnobotanic solutions or self-treatment following wildlife bites and scratches, and may be potential entry point to target audiences to implement future zoonotic disease prevention efforts.

When asked about individual consumption of wildlife, many respondents described how they ate any kind of animal from the forest, or any of the wildlife with which they worked. Among those who specified the kinds of animals they ate, rats, fish, bats, and wild boar were included. Several of the respondents shared that they continued to hunt and consume protected wildlife, such as anoa and yaki, despite being aware of the laws regarding those protected species.

These behavioral risk investigations at Sulawesi bushmeat markets were an immense success made possible by the local team's professional expertise to facilitate nuanced and sensitive discussions. The insights obtained will provide a valuable body of evidence for researchers and practitioners looking to build local evidence-based interventions to better protect the health of those living alongside and working in the bushmeat markets in Sulawesi.

QUANTIFYING & MAPPING THE WILDLIFE TRADE IN SULAWESI

To further explore zoonotic disease risk in the wildlife trade in Indonesia, we used field surveys, ethnographic interviews, and daily counts in markets to document terrestrial wildlife species for sale in North Sulawesi markets, and to identify the hunting sites, practices, and key actors within the trade. These data were collected to better understand the potential for exposure and risk for disease emergence along the wildlife value chain in Sulawesi, the species traded, occupations and behaviors of those involved in the trade, and volume of the trade. We quantified the volume of wild meat traded and the prices of items (to better understand socio-economic drivers associated with disease risk), with a particular focus on the flying fox trade. Wildlife meat was routinely available for sale in 73% of the markets and supermarkets surveyed in North Sulawesi. The wildlife taxa most commonly found in these markets were flying foxes, wild pigs, rats, and snakes. Wildlife hunting and trade networks extend to all provinces on the island through a well-organized, dynamic, and easy to access network involving many actors. We identified 45 flying fox roosts in Sulawesi, 38 of which were under active hunting pressure. A third of the active hunting sites are located in Southeast Sulawesi, which acts as a hub for bat hunting and trade – and a site of greatest risk where bat hunters are in direct contact with captured, live animals. We estimate that the number of flying foxes annually traded in Sulawesi ranges from 662,551 to more than one million individuals and conclude that current rates of flying fox harvest are unsustainable for the population. Stricter law enforcement, implementation of hunting quotas, and further research efforts are therefore urgently needed to improve the sustainability and biosecurity of the wildlife trade in Sulawesi. For details see: Latinne et al., 2020.

FIGURE 6. *(A) Wild meat for sale at a market in North Sulawesi; (B) Bats, wild pigs and birds for sale at a market in North Sulawesi; (C) Macaque, snakes and wild pigs for sale at a market in North Sulawesi (pictures: Suryo Saputro/Alice Latinne).*

FIGURE 7. *(A) Rat roasting in a North Sulawesi market; (B) Roasted rats for sale at a North Sulawesi market; (C) Roasted bats for sale at a North Sulawesi market; (D) Wild pigs, snakes, and bats and dogs for sale at a North Sulawesi market (pictures: Suryo Saputro/Alice Latinne).*

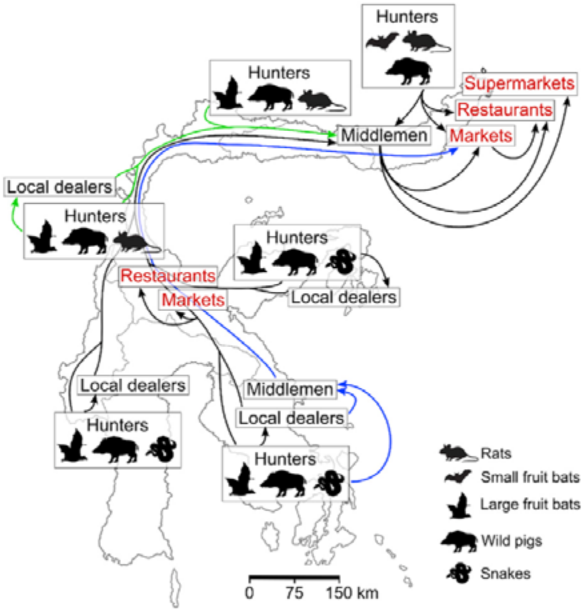


FIGURE 8. *Overview of the wildlife trade network in Sulawesi and key actors involved in trading the four taxa most commonly traded, bats, wild pigs, snakes and rats.*

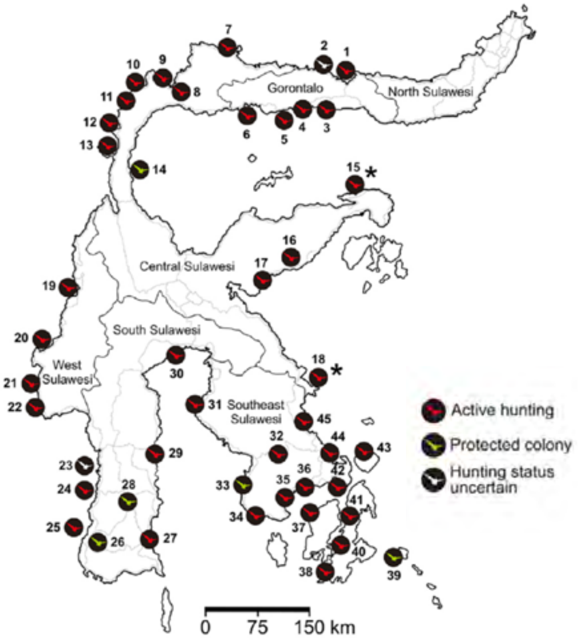


FIGURE 9. *Map of Sulawesi showing the flying fox roosts identified in this study and whether they are hunted. The two* **Pteropus griseus** *roosts in Central Sulawesi are indicated by an asterisk. Other roosts are* **Pteropus alecto** *and/or* **Acerodon celebensis.** *The grey dotted lines corresponds to the provincial road network in Sulawesi.*

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

The PREDICT project in Indonesia facilitated several community meetings and outreach events within communities and health facilities where human surveillance activities were implemented in Sulawesi. Meetings were aimed at providing summaries of surveillance and laboratory findings and sharing resources and strategies to reduce risks for exposure to viruses, while balancing health and conservation goals. To raise community awareness of potential zoonotic diseases, the team promoted safe behaviors and practices related to hygiene, hunting, and wildlife handling. The PREDICT project-developed behavior change and risk communication resource, *Living Safely with Bats*, was one tool used for these activites. This resource was translated into Indonesian in order to be more easily understood by the communities.

CAPACITY STRENGTHENING

The PREDICT team in Indonesia hosted One Health trainings and collaborative workshops with the aim to increase capacity for such work across the country. These trainings ranged from field to laboratory topics and included collaborations with FAO in order to include and work with current professionals and emerging researchers. We also provided training opportunities to strengthen One Health capacity for zoonotic disease surveillance and laboratory testing for the government of Indonesia, including individuals from the Ministry of Environment and Forestry, Ministry of Health, and Ministry of Agriculture. In addition, we trained clinicians and laboratory staff from health facilities in North Sulawesi on biosafety and good clinical practices in biomedical research involving human subjects.

OUTBREAK PREPAREDNESS & RESPONSE

The team in Indonesia supported multiple responses at the request of the government of Indonesia, speaking to the success of the project. These included requests to perform virus group PCR testing from local district health offices, universities, and state-level ministries, as well as providing technical support during outbreak investigations.

PRACTICAL IMPLICATIONS

- We believe a coordinated approach including the communities and local government can result in positive behavior change, and over time improve the health and resilience of community members in the face of zoonotic threats.
- At the policy level, the PREDICT team engaged the local government for promotion and prevention programs associated with understanding zoonotic diseases, and provided input for the development of the Cross-Sector Coordination Guidelines for Zoonotic Outbreaks and Emerging Infectious Diseases in collaboration with Kemenko PMK (Coordinating Ministry for Human Development and Cultural Affairs).
- We also provided technical advice to the national One Health Platform under the Coordinating Ministry of Human Development and Cultural Affairs (Kemenko PMK) on the Sistem Informasi Zoonosis dan Emerging Infectious Diseases (SIZE, EID and Zoonosis Information System) and participated in One Health Assessment for Planning and Performance Multisectoral Workshop organized by the same Ministry in 2018.
- Our data collection on wildlife trade markets in Indonesia showed that hundreds of thousands of bats, wild pigs, snakes, and rats are hunted and traded annually in Sulawesi. These markets have been found to be part of a well-organized and dynamic network involving many actors (Latinne et al., 2020).
- Our in-depth behavioral risk interviews have shown that flying fox hunting is an important source of income in Sulawesi. At the same time, our analysis suggests current rates of flying fox harvests are unsustainable, both from a conservation and food supply perspective (Latinne et al., 2020).
- Through the PREDICT project's investigation of the wildlife trade dynamic, we have found that the trade of protected species is ongoing, albeit at a lower scale. We assert that in North Sulawesi there is an urgent need for stricter law enforcement regarding the protection of theses species. Implementation of hunting quotas, as well as research and conservation efforts are urgently needed to improve the sustainability of the wildlife trade in Sulawesi. Further work and risk-based investigations using a One Health approach such as that utilized by the PREDICT project are needed, as the impact of this trade on Sulawesi local ecosystems remains mostly unknown (Latinne et al., 2020).

FURTHER READING

- Latinne, Alice, et al. "Characterizing and quantifying the wildlife trade network in Sulawesi, Indonesia." *Global Ecology and Conservation* 21 (2020): e00887. DOI: 10.1016/j.gecco.2019.e00887

PREDICT
LAO PEOPLE'S DEMOCRATIC REPUBLIC

ONE HEALTH IN ACTION (2009-2020)

LAO PEOPLE'S DEMOCRATIC REPUBLIC

Investigating viral spillover risk to humans through the wild meat and animal value chain to keep people safe from emerging viruses

Since 2009, PREDICT Lao PDR has worked to better understand the dynamics of how people interact with wildlife through hunting for food or medicines, purchase at wild meat markets, or through incidental contact and exposure in their homes.

In PREDICT-1 (2009-2014), our efforts focused on locating and identifying wild meat markets across the country, and then characterizing the animals available for purchase and the viruses that they carry. In PREDICT-2 (2014-2019), our team focused One Health surveillance in Champasak province, where local communities rely heavily on non-timber forest products to supplement both their income and their diets. Hunting for wild animals such as ground squirrels, giant flying squirrels, and muntjacs is common, with villagers using these animals as valuable sources of protein. Additionally, any wild meat that is not consumed in the village is often sold or traded to nearby wild meat markets, where people from all over the area come to buy live or dead wild animals.

In PREDICT-2, our One Health surveillance incorporated sampling of both humans and livestock to identify viruses that may have jumped from livestock and wild animals into people. By partnering with the Khong District Hospital, the PREDICT project identified individuals who had illnesses that could be characteristic of zoonotic diseases. Detailed questionnaires gathered information about their previous history of illness as well as their exposure to wildlife and livestock. Qualitative, semi-structured interviews gathered even more information and focused on hunting practices within participating villages. A partnership with FAO allowed for the collection of biological samples from livestock populations and to assist in characterizing how people interact with their domestic and food animals.

In addition, the PREDICT team played a crucial role in the training of national, provincial, and district level government staff, Lao PDR's developing One Health workforce, to provide them with skills to better serve their communities and perform their duties. Our laboratory specialists held numerous trainings with national level human and animal health laboratorians, where they learned to perform consensus PCR assays, to prepare samples for genetic sequencing, and about how to keep themselves and others safe while working with biological materials. At the provincial and district levels, our PREDICT team helped teach local veterinary workers how to safely and humanely capture wildlife, collect biological samples, and to collect detailed metadata for future use. Additionally, local staff learned how to safely store biological samples for transport, how to protect themselves while working with animals, and about the diseases that can be shared between animals and people. When performing human surveillance activities, local staff learned more about the mechanisms by which viruses can move between animals and people, how to administer a detailed questionnaire, and techniques on how to perform non-scripted behavioral interviews to gather behavioral risk information from people most at-risk of zoonotic spillover and spread.

Through analysis of project data and findings, the PREDICT project was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

LOCAL PARTNERS

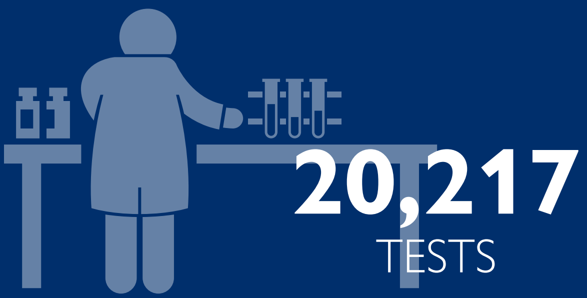
- Bachieng District of Agriculture and Forestry Office
- Champasak Provincial Health Office
- Champasak Provincial of Agriculture and Forestry Office
- Department of Communicable Disease Control
- Department of Livestock and Fisheries
- Food and Agriculture Organization (FAO)
- Khong District Health Office
- Khong District Hospital
- Khong District of Agriculture and Forestry Office
- Ministry of Agriculture and Forestry
- Ministry of Health
- National Animal Health Laboratory
- National Center for Laboratory and Epidemiology
- Soth Village Healthcare Center

DEVELOPED the One Health Workforce by training more than 150 people in Lao PDR.

OPERATIONALIZED One Health surveillance and sampled over 4K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- National Center for Laboratory & Epidemiology
- National Animal Health Laboratory



DETECTED 30 unique viruses in both animal and human populations.

SOUBANH SILITHAMMAVONG

Country Coordinator, *Metabiota*

"The PREDICT program has been a school of its own for me, from which I gained knowledge from both global and local teams of experts. Because of PREDICT, I have been given such a great opportunity to be a part of a program that has given me extensive expertise and the chance to grow my career that I had never had in my life. With almost ten years working on PREDICT, I have learned a lot from experts and practitioners who I have met and interacted with. I am very appreciative and give my sincere thanks to USAID for the last ten years."

SENGXAY PHONTHASY

Laboratory Technician, *Dept. of Livestock & Fisheries, Ministry of Agriculture & Forestry*

"The most valuable experiences I have gained from working on the PREDICT project was learning new techniques for preparing samples for testing, performing conventional PCR assays, and accurately reading PCR test results. PREDICT has supported Lao PDR in terms of technical assistance, equipment, and field and lab supplies for One Health surveillance. Because of this, PREDICT has made me knowledgeable of how wildlife diseases can be transmitted and threaten livestock and human health. If I have the opportunity in the future, I would like to continue my studies at the Master and PhD level, to be able to better serve this country, especially in the areas of early disease detection and quickly responding to outbreaks."

ACHIEVEMENTS

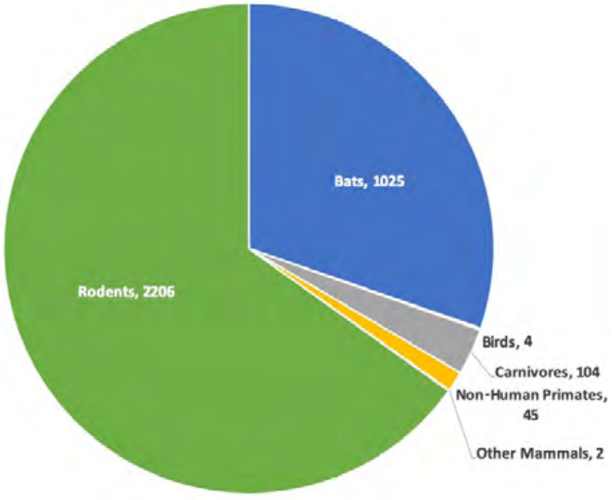
- PREDICT is the first program that has supported the Lao PDR government in building capacity for zoonoses surveillance in wildlife.
- Since 2009, the PREDICT project has conducted disease surveillance in the wild meat value chain and on strengthening lab capacity for detection of viral threats.
- The PREDICT team expanded surveillance of the wildlife value chain to include upstream sources of wildlife harvesting, and implemented One Health surveillance and behavioral risk investigations, providing a platform for multi-sectoral collaboration between the animal and human health sectors.
- PREDICT is a proof-of-concept that wildlife, livestock, and human sectors can work together harmoniously to strengthen health security.
- While outside funding and foreign support remain important for sustaining gains, the PREDICT project has strengthened zoonotic disease surveillance and national laboratory system capacity empowering more independent and nationally-led disease surveillance and outbreak response efforts and leaving behind a foundation more resilient for overcoming current and emerging challenges.

Soubanh Silithammavong, the PREDICT/Lao PDR Country Coordinator, discusses methods for administering a questionnaire for gathering health and animal exposure information with nurses and doctors from the Khong District Hospital.

PHOTO: DAVID MCIVER

CAPACITY STRENGTHENING

In Lao PDR, PREDICT was the first initiative to successfully facilitate the implementation of One Health surveillance, bringing together the animal and human health sectors to launch simultaneous disease surveillance activities in human, livestock, and wildlife populations. For 10 years, the PREDICT project has provided capacity strengthening opportunities and trainings to the national health workforce, paving the way to fully integrated One Health surveillance capabilities. By using a common set of training materials, safety measures, and laboratory techniques, members of both the human and animal health authorities are now prepared to share data and information, assist and complement zoonotic disease surveillance, and collaborate during outbreak response efforts. Additionally, PREDICT was an active contributor to the first ever World Health Organization Joint External Evaluation to take place in Lao PDR, and provided technical assistance to the Lao PDR government supporting gains in disease management capability. Finally, the PREDICT project's achievements and recommendations are supporting local and international stakeholders in identifying future opportunities and areas for further investment for strengthened global health security.



Number of animals sampled by taxa

(Opposite page, top-bottom) The PREDICT/Lao PDR animal sampling team safely takes biological samples and body measurements from rodents that are for sale in a bushmeat market in Champasak Province (credit: Soubanh Silithammavong); PREDICT/Lao PDR team meets with local villagers each time they arrive in the community, to keep them up to date and engaged on the project's progress (credit: Soukkanya Athitang); animals like this giant flying squirrel are commonly found in wild meat markets in Lao PDR. Some are bought for food, while others, like this squirrel, will probably be used for making local medicines from its bones.

PHOTO: DAVID MCIVER

ONE HEALTH SURVEILLANCE

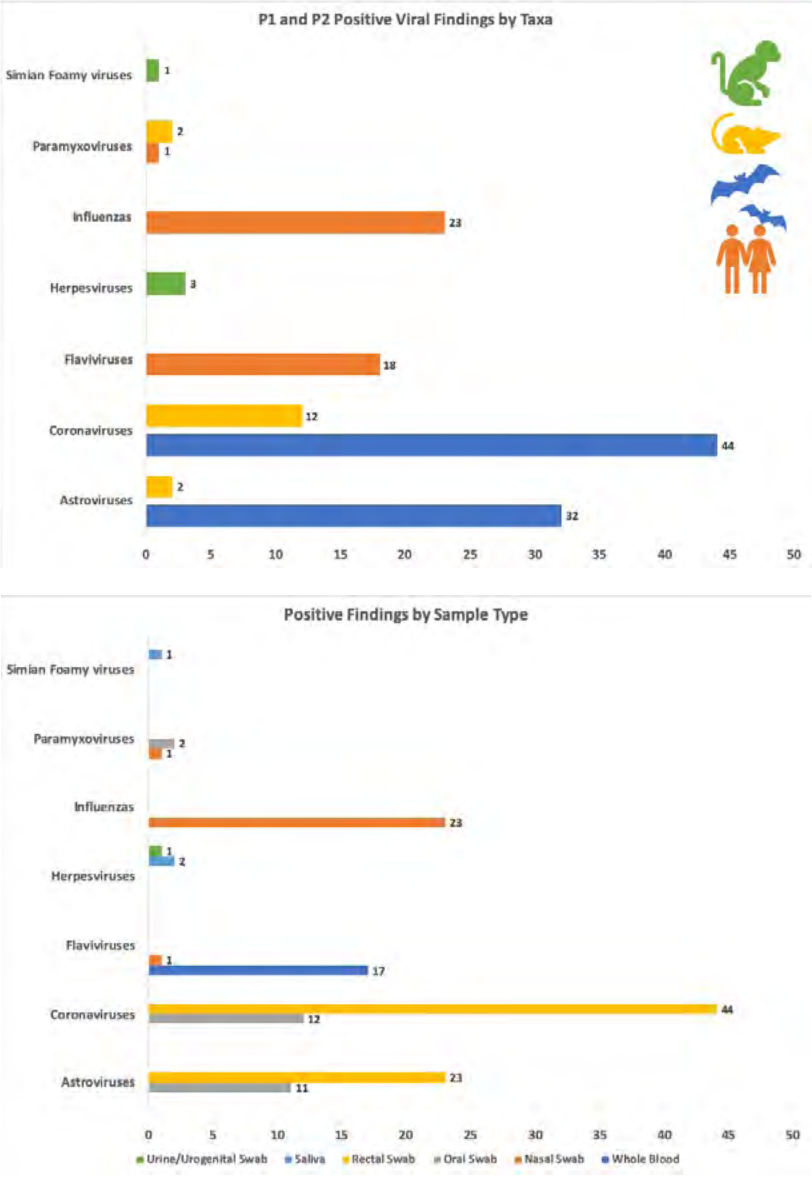
The PREDICT Lao PDR team led highly successful scoping visits and active surveillance activities in close partnership with both human and animal health ministries, and with the Food and Agriculture Organization (FAO). This partnership allowed for the simultaneous input of information and experiences from individuals from the human health sector, wildlife sector, and livestock sector, enabling a more complete perspective of the potential disease transmission environment in these at-risk communities. In partnership with FAO, the PREDICT team was able to identify key locations where there is significant interaction between all animal and human populations, enabling investigations into their relationships and potential zoonotic disease transmission dynamics. While the PREDICT team discussed with village hunters, butchers, and wild meat transporters about the hazards of handling wild animals, the FAO team held discussions with livestock owners and farmers about ways to keep themselves healthy while continuing to raise healthy livestock.

COMMUNITY ENGAGEMENT

PREDICT Lao PDR is committed to community engagement and worked with local villages and partners to raise awareness of zoonotic disease threats and strengthen capacity for prevention, surveillance, and detection. Sensitization meetings were held in all PREDICT project sites over the life of the project. At these meetings, our team introduced the One Health approach and provided a platform for learning and dialogue about risky behaviors and practices associated with viral spillover and spread. In addition, PREDICT provided summaries of project findings and shared resources and strategies to prevent infection and live safely with wildlife.

VIRUS DETECTION

PREDICT's virus detection uses broadly reactive consensus (genus/family level) PCR supplemented with high throughput sequencing. These powerful tools produce specific, high-resolution data, allowing for detection of known and new potential pathogens. In Lao PDR, our laboratory team detected viral RNA in 14 rodents that were either for sale in markets or hunted in villages. In 12 of these animals, coronaviruses closely related to known isolates from rodents were confirmed by sequencing. In the two additional animals, unique and novel paramyxoviruses were sequence confirmed, viruses only distantly related to other currently known rodent paramyxoviruses. Viral RNA from known viruses was also detected and sequence confirmed in 41 humans. Most of these individuals, patients at hospitals where the PREDICT team conducted syndromic surveillance for febrile illnesses, were either positive for Influenza A or dengue virus, with one individual showing co-infection of both. The dengue virus serotypes detected were serotypes 2 and 4. In one patient, our team detected viral RNA of the human parainfluenza virus 2.



Viruses detected by taxa (upper) during PREDICT-1 and PREDICT-2 (2009-2019); unique viruses detected according to sample type (lower; 2009-2014)

VIRUS TABLE

				# OF POSITIVE INDIVIDUALS		
VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	TOTAL	WET SEASON	DRY SEASON
Coronavirus	Longquan Aa mouse coronavirus	Indochinese Ground Squirrel, Polynesian Rat, Red-Cheeked Squirrel	Khong	11	0	11
	Murine coronavirus	Indochinese Ground Squirrel	Khong	1	1	0
Paramyxovirus	Human parainfluenzavirus 2	Human	Khong Hospital	1	0	1
	PREDICT_PMV-151	Finlayson's Squirrel	Khong	1	0	
	PREDICT_PMV-153	Phayre's Flying Squirrel	Khong	1	0	1
Influenza virus	Influenza A	Human	Khong Hospital	23	8	15
Flavivirus	Dengue virus serotype 2	Human	Khong Hospital	4	4	0
	Dengue virus serotype 4	Human	Khong Hospital	14	12	2
Total				**55***	**24***	**31**

*Numbers do not total due to one individual having a co-infection with two viruses

Dr. Bounsavane Dounangboubpha, from the National University of Laos (center), leads the PREDICT animal sampling team deep into the Lao forests to demonstrate the best locations for trapping bats that visit nearby villages in the evenings.

PHOTO: SOUBANH SILITHAMMAVONG

BEHAVIORAL RISK, & RISK COMMUNICATION

Community engagement and outreach are critical for effective prevention, detection, and response to disease threats. When working with community stakeholders, PREDICT held community meetings at the local temple to inform residents of plans and to provide updates on project progress and success. These participatory meetings empowered our communities to learn about zoonoses, One Health, and disease detection and prevention. To supplement data collection, our PREDICT team recruited villagers to help provide in-depth knowledge on the social and behavioral risks that may be associated with viral spillover and spread. We conducted semi-structured interviews and focus groups with people reporting routine wildlife and/or livestock contact targeting key at-risk populations (e.g., wildlife hunters/trappers/traders, wildlife market workers, and livestock farmers). In addition, questionnaires were administered to healthy individuals from the Soth Village Cluster with routine animal contact, and with sick individuals recruited during syndromic surveillance at Khong District Hospital. In total, 13 individuals completed a semi-structured interview, nine individuals participated in a focus group, and 234 individuals completed questionnaires, 34 from the community and 200 from the hospital.

Locations of One Health surveillance and behavioral risk interviews

PRELIMINARY FINDINGS

Our team continues to analyze biological and behavioral risk data to learn more about risks for zoonotic disease transmission in targeted communities. As of this report, our preliminary findings indicate that reported contact with animals was very common among both community and hospital groups. Among the community group, 100% reported contact with animals in the last year; among the hospital group, 99% reported contact with animals in the last year. In the community group, 53% reported slaughtering animals in the last year, while in the hospital group, only 28% reported recent slaughtering. Of those who reported slaughtering animals in the last year, many said they did not know if there were risks associated with slaughtering or butchering with an open wound (67% community vs. 36% hospital). 54% of the hospital group thought there was some risk associated with slaughtering or butchering with an open wound, compared to only 17% of the community participants.

Reported contact with bats, non-human primates, and rodents/shrews was somewhat uncommon. Among the community group, 3% (n=1) reported contact with bats, 3% (n=1) reported contact with non-human primates, and 18% (n=6) reported contact with rodents/shrews within the last year. Among the hospital group, 4% (n=7) reported contact with bats, 1% (n=2) reported contact with non-human primates, and 9% (n=18) reported contact with rodents/shrews within the last year. Among the community group, 3% (n=1) reported hunting/trapping bats and 3% (n=1) reported hunting/trapping rodents/shrews within the last year; among the hospital group, 1% (n=1) reported hunting/trapping bats, 2% (n=4) reported hunting/trapping rodents/shrews, and 1% (n=1) reported slaughtering rodents/shrews within the last year.

QUALITATIVE RESEARCH

Thirteen semi-structured interviews and one focus group were conducted with villagers in the Soth Village Cluster reporting animal contact. Most villagers reported rice production and/or livestock rearing as their primary occupation. Consumption of raw blood was common, but not universally practiced.

Villagers reported that the majority of wildlife hunting/trapping conducted in the neighboring forest was subsistence oriented: animals were primarily captured as a supplemental protein source. While some hunting/trapping did lead to sale at market, this was said to be an exception. Most villagers reported using traps to capture small animals, including flying squirrels, tree shrews, and monitor lizards. The majority of these types of animals were reported dead upon retrieval; consequently, few hunters/trappers reported animal bites or scratches. A minority of hunters/trappers reported using guns to hunt muntjac. Villagers frequently explained that the hunting of larger animals was more common in the past, as populations of animals like primates have dramatically decreased in recent years. Many of the hunter interviews said that if the primate populations were to return, they would once again hunt them. On multi-day trips, some hunters/trappers reported butchering captured animals in the forest and drying the meat before bringing it back. Personal protective equipment (PPE) was rarely reported being used during hunting or butchering.

RISK IDENTIFICATION

- Use of PPE such as leather gloves, proper footwear, or dedicated clothing were rarely used by hunters capturing wild animals. While in these resource poor settings it might be difficult for people to have this type of equipment available, it was found that many people were unaware of the potential for disease transfer between animals and humans by cuts, bites, or scratches, so providing information on how best to protect themselves is important.
- The consumption of raw blood appeared to be a fairly common practice. The risks associated with the consumption of raw/undercooked blood and meat should be communicated and methods for killing any microorganisms in the blood should be discussed.
- Though bat hunting was not a common practice in the villages focused on by PREDICT, people did report occasional interactions with bats in crop fields or in homes. The PREDICT-created book *Living Safely With Bats* will be presented and discussed with communities to increase their awareness of the potential dangers of living with bats, and how they can keep themselves safe from exposure while at the same time ensuring the health and safety of the bats.

PREDICT's* Living Safely With Bats *risk reduction and behavior change communication resource was shared with local communities to increase their awareness of the importance of bats to the ecosystem, and how to be safe and prevent infection when interacting with them.

PRACTICAL IMPLICATIONS

The PREDICT project's work in Lao PDR has been pivotal in establishing the premise of a One Health platform in the country. While Lao national labs are well-established, the PREDICT virus family-level assays brought important disease detection capability and new tools for virus discovery to the country. PREDICT brought into focus the importance of zoonotic disease spillover in Southeast Asia, a hotspot of infectious disease emergence. With its emphasis on capacity strenghtening, the PREDICT project has reinforced national human and animal health preparedness and response capabilities, and through close working partnerships and collaborations across the global PREDICT Consortium, Lao health experts now have access to regional networks enabling sustained collaboration, consultation, and cooperation. This rich multidisciplinary, transborder fabric empowered by PREDICT will persist, we hope, in strengthening national and global health security through continued vigilant surveillance of zoonotic disease threats.

As we say in Lao:

ເຮັດວຽກຮ່ວມກັນ, ພວກເຮົາປະສົບຜົນ ສຳ ເລັດ

[Working together, we succeed]

PREDICT
MALAYSIA

ONE HEALTH IN ACTION (2009-2020)

The PREDICT project in Malaysia is an exemplary One Health success between EcoHealth Alliance and the Malaysian Government (human, wildlife and veterinary authorities) working together to find new viruses and strengthen human and laboratory capacity, an important step towards sustainable disease surveillance.

The PREDICT project, as part of USAID's Emerging Pandemic Threats program, seeks to discover zoonotic viruses in wildlife before they become human epidemics, and identify the factors that drive their emergence, amplification, and spread in populations. EcoHealth Alliance (EHA) and Conservation Medicine, the Ministry of Health, Department of Wildlife and National Parks Peninsular Malaysia, and the Department of Veterinary Services have been working together on zoonotic disease surveillance in Malaysia since 2005. Expanding to Sabah in 2012, EHA, Sabah Wildlife Department, and Sabah State Health Department began a collaboration on zoonotic disease surveillance. This close working and committed group of individuals and institutions have made significant advances in Malaysian research and surveillance infrastructure and have strengthened technical capacity in that time, including the creation of two internationally-certified labs dedicated to wildlife disease screening. In Malaysia, the project to date has identified 76 novel viruses and 29 known viruses, providing the Malaysian government with actionable data to inform risk mitigation policies at the national and state level.

LOCAL PARTNERS

BCI	Bio Community Initiative
BMHRC, UMS	Borneo Medical and Health Research Centre, Universiti Malaysia Sabah
BSBCC	Bornean Sun Bear Conservation Centre
DGFC	Danau Girang Field Centre
DHRU, UMS	Development and Health Research Unit, BMHRC, Universiti Malaysia Sabah
DVS	Department of Veterinary Services
DWNP	Department of Wildlife and National Parks Peninsular Malaysia
FVM, UPM	Virology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia
KKPHL	Kota Kinabalu Public Health Laboratory
MoH	Ministry of Health
MZL, NWFL	Molecular Zoonosis Laboratory, National Wildlife Forensic Laboratory
NPHL	National Public Health Laboratory
NWFL	National Wildlife Forensic Laboratory
SORC	Sepilok Orangutan Rehabilitation Centre
SSHD	Sabah State Health Department
SWD	Sabah Wildlife Department
CTED, FM, UNIMAS	Universiti Malaysia Sarawak
VRI	Veterinary Research Institute
WHGFL	Wildlife Health, Genetic and Forensic Laboratory
WHU	Sabah Wildlife Health Unit
WRU	Sabah Wildlife Rescue Unit

(Opposite page)

PHOTO: TOM HUGHES, ECOHEALTH ALLIANCE/CONSERVATION MEDICINE

662

DEVELOPED the One Health Workforce by training 662 people in Malaysia.

>7.3K

OPERATIONALIZED One Health surveillance and sampled over 7.3K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Wildlife Health, Genetic & Forensic Laboratory, Sabah Wildlife Department
- Virology Lab, Faculty of Veterinary Medicine, Universiti Putra Malaysia
- DWNP Molecular Zoonosis Laboratory at the National Wildlife Forensic Laboratory
- National Public Health Laboratory
- Kota Kinabalu Public Health

>108K
TESTS

76
PREDICT

29
KNOWN

DETECTED 105 unique viruses in both animal and human populations.

NUR AMIRAH MD SUNGIF

Laboratory Technician, *EcoHealth Alliance*

"The experiences that I gained while working on the PREDICT project enabled me to enhance my laboratory and field techniques. Good teamwork is actually the key to how we managed to complete this project successfully and I am happy I am part of this team."

ANDREW GINSOS

Team Leader, *Wildlife Health Unit/Sabah Wildlife Department*

"It was great to work with PREDICT, I really enjoyed the challenging work and learnt a lot through the project. I am amazed at how much work was done by the team to enhance biosafety and develop a surveillance network in Malaysia."

ACHIEVEMENTS

- The PREDICT project in Malaysia has trained 662 people in the last 10 years. Throughout the second phase of the PREDICT project (PREDICT-2; 2014-2019), a total of 532 people have been trained - 224 people in Peninsular, 212 people in Sabah, and 49 people in Sarawak (47 individuals from Peninsular and Sabah were only partially trained, as they did not complete training or failed the quizzes). Most received multiple trainings over the 10 years with 478 from PREDICT-2 being trained in two or more PREDICT project modules. These trainings helped build capacity and ensure the competency and safety of lab and field teams during project surveillance and beyond.
- To improve our understanding of pathogen circulation and exposure risks, the PREDICT Malaysia team conducted concurrent animal-human sampling in 9 Orang Asli villages. Behavioral surveys were conducted to identify perceptions and practices around wildlife, domestic animals, hunting, and livelihood. Working with our partners from the Ministry of Health (MoH) District Health Teams, the Department of Wildlife and National Parks (DWNP), and the Department of Veterinary Services (DVS), we sampled 1,390 people, 736 wild animals and 696 domestic animals and pets – a huge sampling effort that helped strengthen the One Health partnership between MoH, DWNP, and DVS.
- To date, this work with the Orang Asli has found two novel coronaviruses in 3 bats, 4 known coronaviruses in 15 people, one known influenza in one person, one known coronavirus in two chickens, and one known coronavirus in a rat. Serological screening of people and wildlife in Orang Asli communities is being conducted. Initial results suggest that there has been exposure to Nipah-related & Ebola-related viruses in bats, non-human primates (NHP), and indigenous communities. This ongoing work will build from PREDICT and continue to improve our understanding of zoonotic viral spillover risk in communities living in close association with wildlife.
- The PREDICT Malaysia team conducted trainings within four communities in Sabah and nine in Peninsular Malaysia on how to live safely with bats, using the PREDICT project developed resource *Living Safety with Bats*. 829 people attended these community meetings, 95 bat books were provided in Malay, and 10 were provided in the Dusun language to community leaders and the local schools as leave behind material that can continue to be shared with the communities. The PREDICT Malaysia team continues to receive requests and find uses for the developed material, and is preparing *Living Safely with Bats* in the Murut language as an aid for local meetings with communities that rely on subsistence hunting in southern Sabah near the border with Kalimantan.

(Opposite)
PHOTO: JIMMY LEE, ECOHEALTH ALLIANCE/CONSERVATION MEDICINE

ONE HEALTH SURVEILLANCE

Throughout PREDICT-2 (2014-2019), the project focused on zoonotic disease surveillance at high-risk interfaces such as Peninsular Malaysia's Orang Asli communities (many of which practice subsistence hunting of wild animals) and those living or working in areas with lots of wildlife contact and conflict in Sabah Malaysia. PREDICT-2 performed concurrent human, wildlife, and domestic animal/livestock surveillance and identified human behaviors contributing to transmission risk. In addition to this targeted surveillance at high-risk interfaces, hospital-based surveillance was also conducted. In the state of Sabah, some of the indigenous groups still practice traditional, and in many cases subsistence hunting of wild animals, while land use change results in increasing contact and conflict between humans and wildlife. Every year across Sabah patients die in clinics and hospitals across the state from diseases of unknown origin. Of the 76 novel viruses discovered through the PREDICT project to date in Malaysia, 65 were found in Sabah. The use of hospital-based syndromic surveillance could begin to identify the cause of some of these undiagnosed deaths and potentially improve healthcare and outcomes for patients in the future. This effort will continue post-PREDICT.

EcoHealth Alliance Malaysia laboratory coordinator Mei-Ho Lee processing samples at Diagnostic Laboratory of the Department of Wildlife and National Parks.

PHOTO: TOM HUGHES, ECOHEALTH ALLIANCE/CONSERVATION MEDICINE

In 2016, the PREDICT Malaysia team began zoonotic disease surveillance of the Orang Asli indigenous communities in Peninsular Malaysia. These communities employ traditional methods of subsistence hunting of wild animals including bats, rodents, non-human primates, and other mammals, and raise domestic animals such as chickens and hunting dogs around their homes. These communities are often in heavily forested remote areas with limited access to medical and health services. As a result of the high degree of contact with wild and domestic animals through hunting and rearing practices, they are vulnerable to infection with zoonotic pathogens. The remote locations of these Orang Asli communities prevent or lengthen the duration for the identification and treatment of these pathogens.

In June 2018, concurrent sampling at Orang Asli villages across Peninsular Malaysia kicked back into full swing. The PREDICT Malaysia team, working side by side with our partners from the District Health Teams of Gua Musang, Kuala Lipis, Kuala Kangsar, and DWNP, battled raging rivers, deep mud, collapsed bridges, and sickness to enroll, administer the questionnaire, and sample 1,013 people, 381 wildlife, and 383 domestic animals in just 5 months! In just two dry seasons we sampled a total of 1,390 people, 736 wild animals, and 696 domestic animals and pets. This huge sampling effort helped strengthen the One Health Partnership between MoH, DWNP, and DVS, and provided an opportunity to talk to over 2,000 Orang Asli villagers at community meetings about zoonoses, the One Health concept, and the risks posed by contact with wildlife and how to minimize these risks, such as not touching dead wildlife. The PREDICT project also provided a meal to over 1,200 Orang Asli villagers (many who are under nourished) who attended community meetings or were enrolled in the study.

VIRUS TABLE: PREDICT-2

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Alphacoronavirus NL63	Human	Kuala Kangsar	2	2	0
	Betacoronavirus 1 (OC43)	Human	Kuala Kangsar	4	4	0
	Coronavirus 229E (Human strain)	Human	Kuala Lipis	1	1	0
	Human coronavirus HKU1	Human	Kuala Kangsar, Gua Musang, Kuala Lipis	8	8	0
	PREDICT_CoV-52	Diadem Roundleaf Bat, Fawn-Coloured Roundleaf Bat, Horsfield's Myotis	Pristine 3, Semi-Disturbed 1, Semi-Disturbed 2	5	5	0
	PREDICT_CoV-76	Fawn-Coloured Roundleaf Bat, Dark-Tailed Tree Rat	Batu Supu, Semi-Disturbed 1, Semi-Disturbed 3	5	5	0
	PREDICT_CoV-78	Accuminate Horseshoe Bat, Arcuate Horseshoe Bat, Clear-Winged Woolly Bat, Creagh's Horseshoe Bat, Fawn-Coloured Roundleaf Bat, Lesser Short-Nosed Fruit Bat, Trefoil Horseshoe Bat, Unidentified Rhinolophus Bat, Black Rat, Horse-Tailed Squirrel, Long-Tailed Giant Rat, Müller's Giant Sunda Rat, Red Spiny Rat, Whitehead's Spiny Rat	Telupid Pristine 2, Telupid Pristine 3, Telupid Semi-Disturbed 1, Telupid Semi-Disturbed 2, Telupid Disturbed 1, Telupid Disturbed 2, Pristine 1, Pristine 2, Semi-Disturbed 1, Semi-Disturbed 3, Disturbed 1, Disturbed 2, Gua Madai	24	16	8
	PREDICT_CoV-84	Creagh's Horseshoe Bat	Pristine 3, Semi-Disturbed 1, Semi-Disturbed 2	3	3	0
	PREDICT_CoV-87	Dawn Bat, Indian Pipistrelle, Malayan Slit-Faced Bat	Kuala Kangsar, Putrajaya	4	4	0
	PREDICT_CoV-101	Hardwicke's Woolly Bat	Telupid Disturbed 3	1	0	1
	PREDICT_CoV-104	Lesser Bamboo Bat	Kuala Kangsar	1	1	0
	PREDICT_CoV-105	Creagh's Horseshoe Bat	Gua Madai	1	1	0
	PREDICT_CoV-110	Arcuate Horseshoe Bat, Creagh's HorseshoeBat	Gua Madai, Pristine 1	4	2	2
	Philippines/Diliman1525G2/2008	Horsfield's Fruit Bat, Temminck's Tailless Fruit Bat	Telupid Semi-Disturbed 2, Telupid Semi-Disturbed 3	2	1	1
	Murine coronavirus	Dark-Tailed Tree Rat, Müller's Giant Sunda Rat, Unidentified Niviventer Rat, Whitehead's Spiny Rat	Telupid Semi-Disturbed 1, Telupid Semi-Disturbed 3, Telupid Disturbed 3, Disturbed 1, Kuala Lipis	6	4	2
	Infectious bronchitis virus (IBV)	Domestic Chicken	Gua Musang, Kuala Kangsar	2	2	0
Paramyxovirus	Human parainfluenzavirus 2	Human	Hospital Queen Elizabeth	1	0	1
	PREDICT_PMV-72	Creagh's Horseshoe Bat, Fawn-Coloured Roundleaf Bat	Semi-Disturbed 1	2	2	0
	PREDICT_PMV-74	Fawn-Coloured Roundleaf Bat	Pristine 2, Pristine 3	2	1	1
	PREDICT_PMV-81	Fawn-Coloured Roundleaf Bat	Semi-Disturbed 1	5	5	0
	PREDICT_PMV-98	Fawn-Coloured Roundleaf Bat	Pristine 3	1	1	0
	PREDICT_PMV-99	Fawn-Coloured Roundleaf Bat	Pristine 3	1	1	0
	PREDICT_PMV-105	Creagh's Horseshoe Bat	Semi-Disturbed 1	1	1	0
	PREDICT_PMV-106	Creagh's Horseshoe Bat	Pristine 1, Semi-Disturbed 2	2	2	0
	PREDICT_PMV-107	Arcuate Horseshoe Bat, Creagh's Horseshoe Bat	Pristine 3	2	2	0
	PREDICT_PMV-108	Diadem Roundleaf Bat	Pristine 3	1	1	0
	PREDICT_PMV-110	Fawn-Coloured Roundleaf Bat	Semi-Disturbed 2	1	1	0
	PREDICT_PMV-137	Trefoil Horseshoe Bat	Telupid Disturbed 2	1	0	1
	PREDICT_PMV-120	Moonrat	Pristine 2	1	0	1
Influenza virus	Influenza A	Human	Kuala Lipis	1	0	1
Flavivirus	PREDICT_Flavi-6	Diadem Roundleaf Bat	Pristine 3	1	1	0
Total				**96**	**77**	**19**

Mohd Izman Mokthar Kuala Kangsar Medical Assistant drawing blood from a participant

PHOTO: TOM HUGHES, ECOHEALTH ALLIANCE/CONSERVATION MEDICINE

VIRUS FINDINGS IN PEOPLE

Pathogen surveillance has historically been lacking in indigenous communities, and there is a poor understanding of infection rates of known disease, let alone novel zoonotic viruses. Although novel viruses were not detected in people at any of the Orang Asli study sites, the PREDICT Malaysia team identified 15 cases of four unique, previously known human coronaviruses (NL63, OC43, 229E, and HKU1) and one case of influenza A. One known paramyxovirus, human parainfluenza virus 2, from a patient in the hospital-based syndromic surveillance study in Sabah was detected. Despite serological evidence from another ongoing study suggesting possible exposure to one or more henipaviruses and filoviruses in bats and people at the Orang Asli study sites, no henipavirus or filovirus nucleic acid was detected in any human or animal sampled. The presence of antibodies suggests that spillover may have occurred, and may occur in the future, providing valuable information to help government partners develop targeted surveillance and intervention strategies.

VIRUS FINDINGS IN ANIMALS

Throughout the second phase of the PREDICT project (2014-2019), we detected three known viruses and 22 novel viruses in wildlife and domestic animals using the PREDICT testing strategy of consensus PCR assays that detect a broad range of viruses, known and novel, from priority virus groups: coronaviruses, paramyxoviruses, filoviruses, influenza viruses, and flaviviruses.

The three known viruses detected were all coronaviruses: infectious bronchitis virus was found in domestic chickens, murine coronavirus was found in several rodent species, and Philippines/Diliman1525G2/2008 coronavirus was found in two bat species.

The 22 novel viruses detected were predominately paramyxoviruses (n=12) and coronaviruses (n=9), with one belonging to the flavivirus family. These viruses were found in a diverse array of bat genera, representing at least 14 unique species, as well as within seven unique rodent species, and one moonrat, which despite its name, is a carnivorous mammal not closely related to rodents (Figure 1).

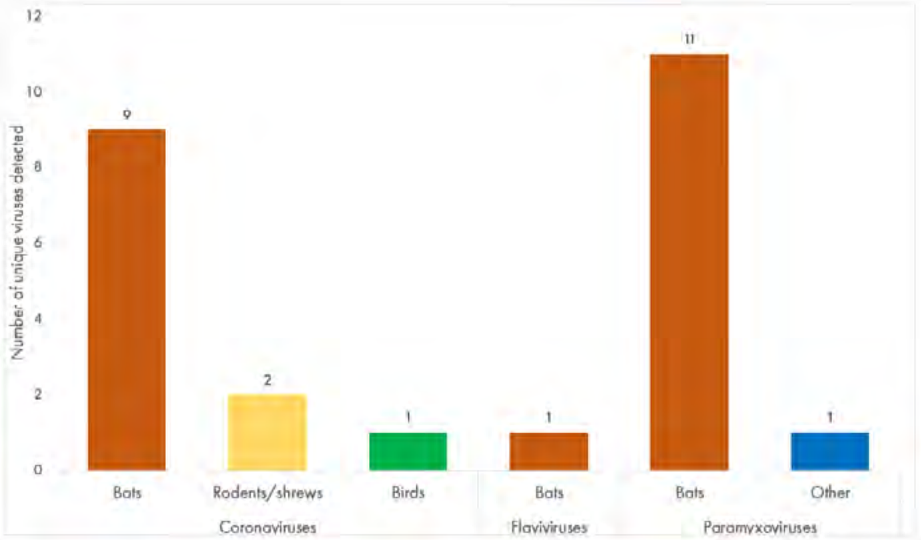


FIGURE 1. ***Summary of known and novel virus detections across major host taxonomic groups***

EPIDEMIOLOGICAL & BEHAVIORAL RISK

EXPOSURE RISKS IN THE INDIGENOUS POPULATION OF PENINSULAR MALAYSIA, THE ORANG ASLI

To gain a more complete understanding of viral spillover risk, the second phase of the PREDICT project expanded the scope to include human data collection concurrently with wildlife and domestic animal sampling. To improve understanding of pathogen circulation and exposure risks, the PREDICT team conducted concurrent animal-human sampling in nine Orang Asli communities. Behavioral surveys were conducted to identify perceptions and practices around wildlife, domestic animals, hunting, and livelihoods. Working with our partners from the Ministry of Health (MoH) District Health Teams (from Gua Musang, Kuala Lipis, and Kuala Kangsar), the Department of Wildlife and National Parks (DWNP) and the Department of Veterinary Services (DVS), we sampled 1,390 people, 736 wild animals (bats and rodents) and 696 domestic animals and pets, including dogs, chickens, goats, wild boars, monkeys and a masked palm civet.

The Orang Asli practice traditional and in many cases subsistence hunting of wild animals and raise domestic animals such as chickens and hunting dogs around their villages. Their health and nutritional status tends to be far worse than the rest of the Malaysian population. Many of these communities have limited access to medical services which means disease spillover events could be going unrecorded. Because of the high degree of contact with wild and domestic animals through hunting and rearing practices the Orang Asli are vulnerable to infection with zoonotic pathogens. Encroachment on their ancestral lands for agriculture expansion and mining leads to increased contact and conflict with wildlife, contamination of their water supplies, and disrupts their traditional way of life, thus increasing their vulnerability. Despite the potential risks for zoonotic pathogen transmission, there are few studies from these communities characterizing behaviors related to animal contact that could influence risk of viral spillover.

The PREDICT team and partners sampled and administered questionnaires to 1,390 Orang Asli individuals, 820 (59%) of which were female and 570 (41%) were male. In total, 119 (8.6%) Orang Asli participants were aged 18-20 years old, 482 (34.7%) were aged 21-30 years old, 346 (24.9%) were aged 31-40 years old, 239 (17.2%) were aged 41-50 years old, 137 (9.9%) were aged 51-60 years old, 46 (3.3%) were aged 61-70 years old, 18 (1.3%) were aged 71-80 years old, and 3 (0.1%) were aged 81-90 years old. Participants from nine communities representing three districts in Peninsular Malaysia (list) were interviewed to investigate the risk of zoonotic diseases based on multiple factors such as livelihood, travel, work, and types of contact with wildlife.

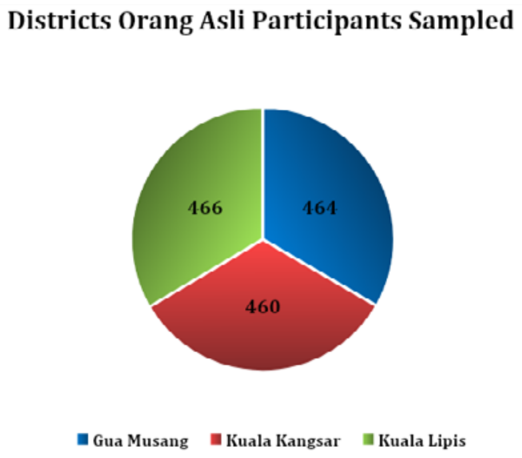


FIGURE 2. *Number of Orang Asli individuals sampled in each district*

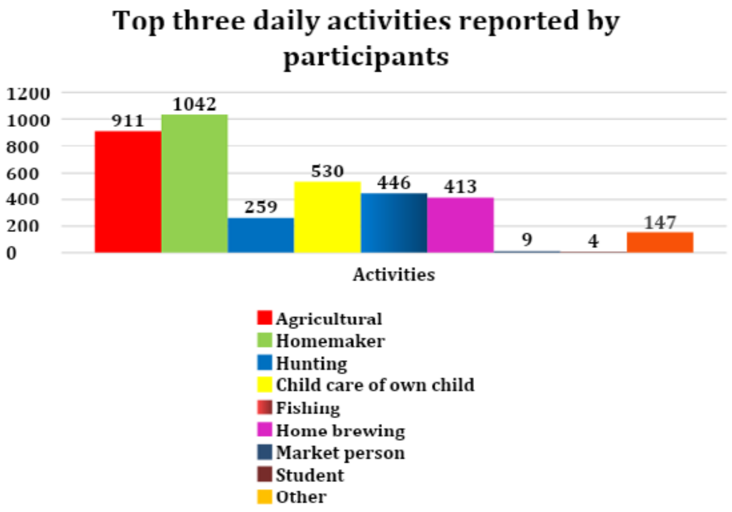


FIGURE 3. *Distribution of Orang Asli participants' top three daily activities*

EcoHealth Alliance Field Veterinarian Dr. Zahidah Zeid collecting samples from a wild boar piglet being kept as a pet in an indigenous village

PHOTO: JIMMY LEE, ECOHEALTH ALLIANCE/CONSERVATION MEDICINE

The PREDICT project provided an opportunity, through its commitment to community outreach in Malaysia, to talk to over 2,000 Orang Asli villagers. They are not entirely isolated from the rest of Malaysia, traveling to more populous and sometimes urban areas for education, work, health care, or to visit other communities. As these communities become increasingly connected through agricultural expansion, urban spread, and an improved road network, they become increasingly important as a sentinel population. Through trust built between PREDICT and the communities, virus surveillance was coupled with education and outreach about the risks associated with wildlife contact. At all community meetings and sampling events the PREDICT team talked to community leaders and members about zoonoses, the One Health concept, and the risks posed by contact with wildlife and how to minimize these risks, such as not touching dead wildlife. Surveillance of Orang Asli communities represents a critically important opportunity to detect spillover of zoonotic viruses. The book *Living Safely with Bats* was used as an education and risk communication tool in some of the indigenous communities who participated in PREDICT project surveillance.

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

Gaining the trust of the Orang Asli communities generated an entry point for continued engagement. Despite the remoteness of many of the sites, the logistical challenges they posed, and the need for multiple visits to each village to meet with community leaders to explain the study and how it would benefit their communities, the team managed in just three dry seasons to do what many thought was not possible. Each sampling trip started with a community meeting and meal to share information about zoonoses and also provided a chance for these communities to get to know the team. Participants were given a health assessment and provided with treatment by the District Health Teams, providing a pathway for improved access to health care. Returning to each community to share results with participants and a summary of project findings strengthened this relationship and provided another opportunity to share health information such as the PREDICT project-developed guide *Living Safely with Bats*. This work demonstrates how One Health novel pathogen surveillance and outreach can be combined with wider public health service delivery for known disease threats to tackle overall health needs.

CAPACITY STRENGTHENING & MALAYSIA ONE HEALTH PARTNERSHIPS

Since 2010, the PREDICT Malaysia team has trained a total of 662 participants (130 in PREDICT-1 and 532 PREDICT-2), increasing the One Health capacity in Malaysia. Training programs provided by the PREDICT project covered both field and lab activities in order to build capacity across the human, wildlife, and livestock sectors for disease surveillance, and to minimize the risks faced by the One Health workforce and students who will join the One Health workforce when carrying out disease surveillance whether in the lab or field. Participants from different backgrounds including senior government staff, scientists, researchers, wildlife conservationists, lecturers, students, rangers, veterinarians, hospital staff, and District Medical teams have received PREDICT project training in surveillance and diagnostics, including the sharing of standard operating procedures (SOPs) and protocols. Many training sessions have been run with participants from different government departments helping to build working relationships and strengthen communication across the human, wildlife, and livestock sectors and between government and academia. Many participants have been involved in multiple ongoing trainings since 2010.

Participants from governmental and non-governmental agencies including MoH, SWD, WRU, WHU, DWNP, DVS, VRI, NPHL, KKPHL, NWFL, BSBCC, DGFC, Lok Kawi Wildlife Park, WWF, KL Bird Park, Kyoto University, Orangutan Appeal UK, Oregon Zoo, Sunway Lost World of Tambun, Myne Resort, Sedafiat Sdn Bhd, Texas Technology University, Selangor Turf Club, UNIMAS, UMS, UPM, Management and Science University, University Tunku Abdul Rahman, University of Queensland Australia, University California- Davis, British Columbia Institute of Technology, Burnaby BC, Iowa State University, Zoo Negara, and Taiping Zoo have joined in the PREDICT project capacity building trainings and utilize the information and skills learned in their daily activities.

Dr. Zahidah Zeid and Ranger Adilah Aziz conducting health check on a Bornean sun bear at Bornean Sun Bear Rehabilitation Centre.

PHOTO: Yuery Wazlan, EcoHealth Alliance/Conservation Medicine

STRENGTHENING THE MALAYSIA ONE HEALTH WORKFORCE: WILDLIFE DEPARTMENTS ENGAGING IN DISEASE SURVEILLANCE AND PANDEMIC PREPAREDNESS

An excellent example that highlights the Government of Malaysia's commitment to One Health and the sustainable capacity developed by the PREDICT project is the establishment and sustained commitment to the Molecular Zoonosis Laboratory at the National Wildlife Forensic Laboratory based at DWNP headquarters in Kuala Lumpur. The BSL-2-certified laboratory, developed in partnership with PREDICT Malaysia conducts all of the wildlife testing for the PREDICT project in Peninsular Malaysia and has expanded to include serology with support from the Defense Threat Reduction Agency's Cooperative Biological Threat Reduction Program. As a sign of the Government of Malaysia's commitment to wildlife surveillance, the Ministry of Natural Resources and Environment has provided operational funding to maintain the lab's infrastructure. In addition, the Wildlife Disease Surveillance Program established by DWNP, which includes the 46 officers from DWNP trained by the PREDICT project, conducts regular wildlife surveillance for the PREDICT project and other disease surveillance projects and outbreak response related to wildlife viruses.

In Sabah, PREDICT Malaysia established the Wildlife Health, Genetic and Forensic Laboratory (a BSL 2 certified laboratory) and the Wildlife Health Unit (WHU) with SWD and DGFC in 2012. The WHU is responsible for leading the physical and diagnostic evaluation of rescued and relocated wildlife across Sabah, as well as conducting sampling trips to trap and sample free-ranging wildlife for the PREDICT project and Deep Forest Project. In 2018-2019, the WHU shone when team leader Andrew Ginsos from SWD WRU helped to organize and run field trips when the PREDICT Malaysia team was deep in the jungle on Peninsular Malaysia and unavailable. Andrew lead the WHU team in Sabah allowing the team to complete the Deep Forest Project as planned. To date, EHA has trained 13 WRU staff who have worked as part of the WHU since 2012. Staff who have spent time in the WHU are now seen as a huge asset to SWD and the WRU by helping to train their colleagues and conduct risk assessments for their various activities not directly related to the PREDICT project, thus ensuring the safety of their colleagues and the wildlife they work with.

The inclusion of wildlife departments in national zoonotic disease surveillance strategies is central to the One Health framework and fundamentally important for cohesion among sectors monitoring zoonotic pathogens in wildlife, livestock, and people. Historically, wildlife departments have rarely been part of public health infrastructure, however, Malaysia has led the way over the last decade in response to the recognition of the importance of wildlife as reservoirs for zoonotic pathogens and the gaps that exist in surveillance systems. Malaysia has been a leader in coordinated government response to zoonotic disease largely as a result of its history with Nipah virus, which emerged in humans from bats via pigs in 1998. Through its partnership with the Government of Malaysia and Sabah State government agencies, the PREDICT project has sustainably strengthened existing surveillance and response systems and paved the way for continued leadership in pandemic preparedness and response.

Disease outbreak response and preparedness capacity was strengthened to support the public health capabilities in Malaysia. Outbreak response training was provided to 25 people in Peninsular and 42 people in Sabah. This training provided an introduction to the theory and skills needed to safely collect samples and work during an outbreak. It included lectures on: Zoonotic diseases of rodents, primates, and bats; Biosafety and PPE; Human safety; Lab safety; Safe animal capture and sampling; Implementing a cold chain for safe sample transport; Packing and shipping biological samples; Trizol training; Risk assessment training; Wet labs on PPE and mask fit test; and a table top exercise preparing front line medical staff on how to deal with an unknown infectious disease outbreak. Additionally, these trainings were bolstered as an additional 53 people in Peninsular and 52 people in Sabah were trained on the PREDICT Emergency Preparedness Module (Figure 5).

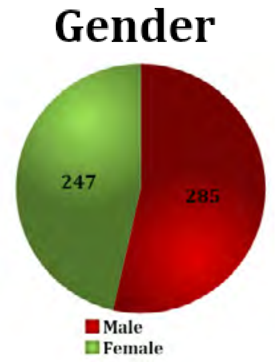


FIGURE 4. ***Number of people trained in One Health throughout the PREDICT project (2014 - 2019) based on gender.***

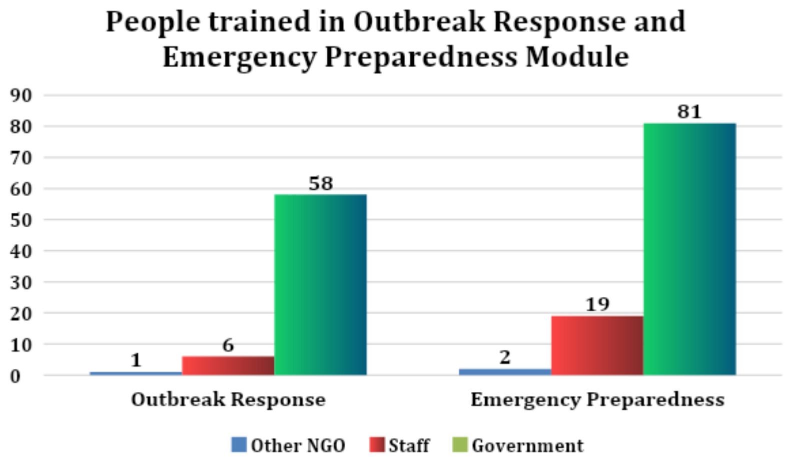


FIGURE 5. ***Number of people trained in Outbreak Response and Emergency Preparedness Module under the PREDICT project from 2014-2019.***

PROMOTING CONSERVATION & STRENGTHENING LAB CAPACITY

Sabah is home to various endangered wildlife species, thus the PREDICT project is working closely with Sabah Wildlife Department and local non-governmental organizations to promote conservation and enhance the lab capacity for disease testing. In order to better understand the health of key wildlife species and the threats faced by zoonosis and anthroponosis, health checks and disease screenings have been carried out by the PREDICT team in collaboration with Sabah Wildlife Department, Sepilok Orangutan Rehabilitation Centre (SORC), Bornean Sun Bear Conservation Centre (BSBCC), and the Wildlife Rescue Unit for the endangered orangutans, sun bears, and Bornean elephants held in captivity as well as from the wild. The PREDICT team sampled 74 orangutans, 64 sun bears, and 13 elephants in Sabah and samples were tested at the WHGFL. This state-of-the-art facility enables wildlife health monitoring, population/conservation genetics research, and wildlife forensic work. It was the first certified BSL-2 level facility in the region dedicated to wildlife work and allows the Sabah Wildlife Department to rapidly assess wildlife health prior to relocation, to engage in comprehensive disease screening efforts, and support research projects. Through PREDICT lab testing, we have found a total of six novel and six known viruses from these three iconic species. The results are essential for Sabah Wildlife Department to make decisions on the further actions required to conserve these important animals.

For example, during an annual health screening for the SORC orangutans, the team from PREDICT and the Wildlife Health Unit collected samples from three quarantined animals, and the WHGFL facilitated the testing of these samples using published Hepatitis PCR protocols. The analysis of sequencing results by Malaysia's PREDICT project Laboratory Coordinator at WHGFL showed that the three orangutans had orangutan Hepatitis B, endemic to wild orangutans which does not pose a public health concern. Soon after that, Tiger, Rosalinda, and Yoda were released into their newly-built rehabilitation cage and began playing on the ground, enjoying the sunshine for the first time ever after being quarantined. Six years later in 2018 one of these magnificent animals, Tiger, was returned to the wild thanks to the efforts of SWD, the staff at SORC, and the Wildlife Rescue Unit.

EcoHealth Alliance Malaysia Laboratory Coordinator Mei-Ho Lee loading samples int Easymag for nucleic acide extraction

PHOTO: SURAYA HAMID, ECOHEALTH ALLIANCE/CONSERVATION MEDICINE

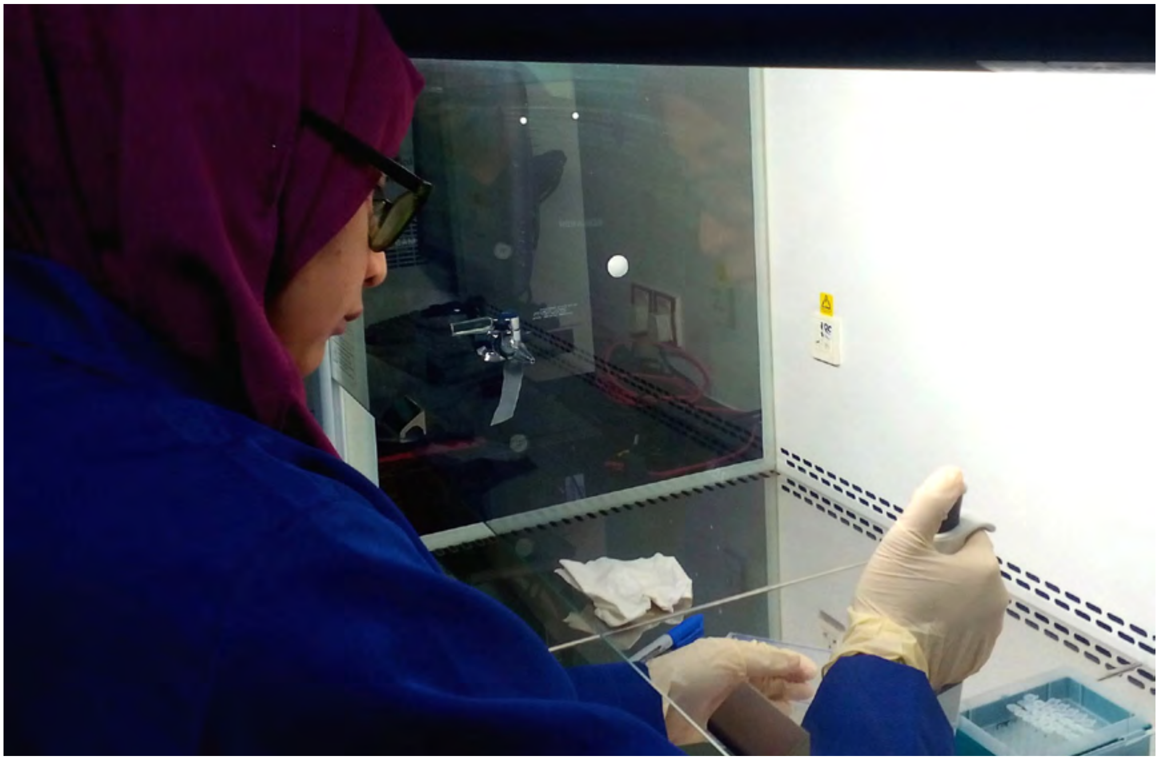
In 2016, three captive orphan elephants died at the rescue center located at SORC. An investigation into elephant deaths led by EcoHealth Alliance's lab coordinator, in collaboration with elephant endotheliotropic herpesvirus (EEHV) experts at WHGFL, found a new and distinct strain of EEHV that caused the infection. This knowledge can help SWD better manage orphaned elephants in captivity and improve disease surveillance in wild populations. Screening of the sun bears found a related virus to ursid herpesvirus 1 from seven sun bears that has not previously been reported in Bornean sun bears. This will help BSBCC prepare to release some of the 44 bears back into the wild. In addition, through PREDICT project activities we have not only discovered new viruses; the Deep Forest Project has allowed us to better understand the impact of land use change on species diversity and population dynamics. The genetic barcoding of sampled animals helped us to better understand the identity of and distributions of these host species.

LAB CAPACITY BUILDING ACROSS MALAYSIA

The PREDICT Malaysia team built laboratory capacity in Sabah in collaboration with SWD and DGFC by establishing the Wildlife Health, Genetic and Forensic Laboratory. In August 2019 the lab was certified for the sixth year in a row as a BSL 2 biocontainment level laboratory in accordance with the Biosafety in Microbiological and Biomedical Laboratories 5th Edition (December 2009) which is the US standard used by CDC and NIH for laboratory specifications. The laboratory was the first certified BSL 2 level facility in the region dedicated to the detection of wildlife pathogens, allowing SWD to rapidly screen wildlife prior to relocation, and to engage in a variety of infectious disease research projects.

The PREDICT Malaysia team built laboratory capacity in Peninsular Malaysia in collaboration with DWNP by establishing the Molecular Zoonosis Laboratory at the new National Wildlife Forensic Laboratory. In May 2019 the lab was certified for the third year in a row as a BSL 2 biocontainment level laboratory in accordance with the US standard used by CDC and NIH for laboratory specifications. This state-of-the-art facility has allowed DWNP to play an even larger role in zoonotic disease research projects.

The PREDICT Malaysia team helped to improve and standardize protocols and SOPs for each partner laboratory in Malaysia, and provided reagents, primers, and a universal positive control that can be used to screen samples for 17 virus families. These primers and controls have been used by NPHL, KKPHL, UPM, and DWNP for disease surveillance outside the PREDICT project.



DWNP Research Officer Millawati adding template for a PCR screening of PREDICT samples at the National Wildlife Forensic Laboratory

PHOTO: MEI-HO LEE, ECOHEALTH ALLIANCE/CONSERVATION MEDICINE

STRENGTHENING ONE HEALTH PARTNERSHIPS

In February 2018, PREDICT Indonesia team members from Bogor Agricultural University, Science Techno Park (IPB-STP) visited DWNP's NWFL to learn about lab design and discuss future collaborations. These individuals included Dr. Diah Iskandriati, Scientific Advisor, Dr. Dadang Syamsul Munir, MM, Director, and Dr. Meika Syahbana Rusli, President Director. IPB-STP is in the process of building an integrated laboratory facility to support its function with some funding from the Indonesian government. One of its purposes will be to provide laboratory services for wildlife. At the moment, Indonesia does not have a centralized laboratory to support analysis for pathogen detection, genetics, and forensics. The purpose of this visit was to learn about laboratory services for wildlife from the established wildlife laboratories in the region (the NWFL being one of the best examples) to apply to their facility, as well as to see the possibility to build networking and collaboration with wildlife laboratories in the region. This visit highlights the importance of regional One Health collaborations which the PREDICT project helped facilitate.

INTEGRATING ONE HEALTH & CONSERVATION

The PREDICT Malaysia Field Manager, Jimmy Lee, was made a member of the International Union for Conservation of Nature and Natural Resources (IUCN) Pangolin Species Survival Specialist Group. In December 2017, he represented the PREDICT project in Malaysia at the One Plan Approach Conservation Planning and Formosan Pangolin PHVA Workshop at the Taipei Zoo in Taiwan. The conference was an opportunity to work with representatives from the Taipei Zoo, Taiwan Forest Bureau, Endemic Species Research Institute, the IUCN SSC, and other organizations to assess potential conservation management plans for pangolins. This opportunity highlights the key work the PREDICT project is doing in the region to strengthen links between conservation and One Health. To further strengthen these goals, he also presented a poster "Surveillance of zoonotic viruses and population genetics of confiscated Sunda pangolins (*Manis javanica*) from Malaysia" at the 5th International One Health Congress in Saskatoon, Canada, June 2018.

In addition to managing all PREDICT project field activities in Peninsular Malaysia and Sabah, Jimmy is also enrolled in a Master's degree program at Universiti Malaysia Sabah conducting pangolin research. Pangolins are critically endangered and the most illegally traded mammal in the world; 1.1 million pangolins were traded between 2006 and 2015. Pangolins are smuggled from countries in Southeast Asia, including Malaysia, to China as a delicacy or for traditional Chinese medicine. This research will provide important information regarding the presence of zoonotic viruses in confiscated pangolins and where these animals were smuggled from. This data will help to lobby governmental agencies and the public to stop the pangolin trade and prevent disease transmission, thus protecting regional economies, public health, and pangolin populations.

PRACTICAL IMPLICATIONS

- The PREDICT project has strengthened the multisectoral platform for partnership around One Health and zoonotic disease surveillance.
- In the last 10 years, the PREDICT Malaysia team has trained 662 current and future One Health workforce individuals.This ensured everyone involved in sampling and testing were fully equipped and capable to safely carry out zoonotic disease surveillance in the field and the lab, adhering to the standards established in PREDICT project protocols.
- The creation of the WHU in Sabah and DWNP's Wildlife Disease Surveillance program guarantees that the wildlife authorities continue to be able to play an important role in zoonotic disease surveillance by identifying novel viruses before they spillover into human and livestock populations. The significant capacity building across all our partners' labs, laboratory screening, and biosafety and field training activities in Malaysia complements all of our government partners' efforts to improve One Health surveillance and capacity building among their staff.
- The establishment of the Molecular Zoonosis Laboratory at DWNP's NWFL and the Wildlife Health, Genetic and Forensic Laboratory for SWD has significantly strengthened virus surveillance capacity in wildlife and resulted in the identification of 76 novel viruses. Noteworthy mentions include the discovery of 12 novel coronaviruses in bats and rodents, some of which are genetically related to SARS-CoV, 15 novel paramyxoviruses in bats and a moonrat, and 1 novel flavivirus detected in a bat. Many of these novel viruses warrant further investigations to determine their pandemic potential. This ongoing effort is a critical step in understanding the risk that wildlife viruses pose to humans and will provide actionable data for our government partners to help keep the Malaysian public safe from emerging zoonotic diseases.
- The PREDICT project has helped Malaysia fully embrace the concept of One Health and recognize the importance of ongoing zoonotic disease surveillance. The continued collaboration between EcoHealth Alliance, Conservation Medicine, and Malaysian government partners will allow for the continuation of efforts to find new viruses and strengthen human and laboratory capacity in-country.

PREDICT
MONGOLIA

ONE HEALTH IN ACTION (2014-2020)

Mongolia's One Health approach to avian influenza surveillance in wild birds

In Mongolia, the PREDICT project focused on improving the national capacity for wild bird surveillance and early detection of avian influenza in order to help address threats to domestic animals and human health. Wild birds are the evolutionary hosts for influenza viruses which cause a range of symptoms in people from mild illness to death. FAO estimates the global economic costs of losses in the livestock/poultry sector from highly pathogenic avian influenza since 2003 are in the billions of US dollars.

Located geographically where three major migratory flyways (East Asian-Australasian, Central Asian, and West Asian-East African Flyway) overlap, Mongolia is of key importance in understanding the role wild birds play in the maintenance and spread of influenza viruses including highly pathogenic avian influenza H5N1. Within Asia, Mongolia is a particularly ideal location to study the dynamics of wild bird influenza virus because the country has very low densities of domestic poultry compared to neighboring countries, supports large concentrations of waterfowl, and has important breeding, molting, and pre-migratory staging areas, thus the relative absence of poultry within the country provides a near unique opportunity to study the epidemiology of the virus in the absence of domestic influence.

MONGOLIA

Mongolia does not have a national or local wildlife authority, and thus lacks a wildlife disease surveillance and outbreak response system. The team worked with four national institutions (two in the human health sector and two in the animal health sector), strengthening the collaborations among Mongolia's existing platforms to address zoonotic diseases by incorporating field-based wildlife disease surveillance, laboratory screening of wildlife samples, and information sharing across One Health partners.

The country first experienced highly pathogenic avian influenza H5N1 outbreaks in 2005 and 2006, following the emergence of the H5N1 clade 2.2 viruses in wild bird populations at Qinghai Lake in China. Mongolia then experienced a lull during 2007 and 2008, when no further outbreaks were detected in China. Then in 2009, a wave of outbreaks in central and western Mongolia occurred, with further outbreaks in Sukhbaatar Province in May 2010 and neighboring Tuva Republic in Russia in June 2010. This new virus, H5N1 clade 2.3.2, that circulated was distantly related to the one that caused earlier outbreaks, and likely represented a spillover from domestic sources, as earlier wild bird cases were detected in Hong Kong (2007/8), Japan (2008), and China (2009).

The movement of wild birds contributes to the global movement of these viruses and there is a need to continue avian influenza monitoring systems in wild bird populations. Monitoring is important to prevent spread of influenza viruses to domestic poultry and from poultry to humans as well as to prevent spill back of highly pathogenic influenza viruses from poultry to wild birds.

In this way, avian influenza was used as a model for building general zoonotic disease capacity in Mongolia and provided an important wild bird surveillance opportunity to prevent and to respond to outbreaks involving wildlife and wild birds.

The PREDICT project engaged at the national level with organizations, centers, and laboratories to implement testing for avian influenza using PREDICT protocols in Mongolia using a One Health approach:

- The National Influenza Center (NIC) - responsible for human health screening and reporting on Influenza Like Illnesses (ILI) throughout Mongolia and providing surveillance, outbreak response, and other laboratory diagnostic guidance to the National ILI system through direct connection to all provincial human hospitals.
- The National Center for Zoonotic Diseases (NCZD): responsible for surveillance, monitoring and reporting of zoonotic diseases of high human concern such as bubonic plague, rabies, anthrax, highly pathogenic avian influenza, emerging and re-emerging zoonotic diseases, and vector borne diseases.
- General Authority for Veterinary Services (GAVS): responsible for National law on livestock and animal health thus oversees the National Strategy and Policy on animal health, guides and implements National level livestock disease surveillance, vaccination, post vaccination immunization and outbreak response. Oversees the State Central Veterinary Laboratory and all 21 provincial veterinary laboratories and their disease response and surveillance capacity.
- The State Central Veterinary Laboratory (SCVL): responsible for all livestock and animal disease diagnosis and confirmation at the National level and has close working relationships with regional and global reference laboratories as well as guides and supports all provincial laboratories in 21 provinces of Mongolia. As a PREDICT partner, SCVL supported field team trainings that included safe sample collection, sample storage, cold chain, and appropriate PPE use. All the samples collected were tested at SCVL and positive samples were subtyped and further tested at the SCVL or partner laboratories.
- The team also worked at the local level engaging provincial level veterinary laboratories, centers for zoonotic disease, protected areas, and local environmental agencies. These partners are important players for One Health field surveillance, monitoring, response actions, and readiness. Annual field-based trainings were held that targeted the provincial level partners to strengthen their response, preparedness, and surveillance capacity. The annual trainings focused on wild bird identification, safe sample collection procedures, cold chain, sample storage, and PPE use, all critical elements of a zoonotic disease surveillance system.

LOCAL PARTNERS

- Environmental Protection Agencies in Dornod, Khovd, and Gobi Atlai Provinces
- General Authority Veterinary Services (GAVS)
- Mongolian Academy of Sciences (MAS)
- National Center for Zoonotic Disease (NCZD)
- National Influenza Center (NIC)
- Protected Area Authorities including Khar Us Lake, Mongol Els, and Dariganga National Park
- Provincial Veterinary Laboratories in Arkhangai, Bulgan, Bayan-Ulgii, Dornod, Khentii, Khovd, Khuvsgul, Sukhbaatar, Uvs, Arkhangai, Bulgan, Bayan-Ulgii, Khentii, Khuvsgul, Khovd, and Uvs Provinces
- State Central Veterinary Laboratory (SCVL)
- University of California, Davis
- USAID
- Wildlife Conservation Society (WCS)

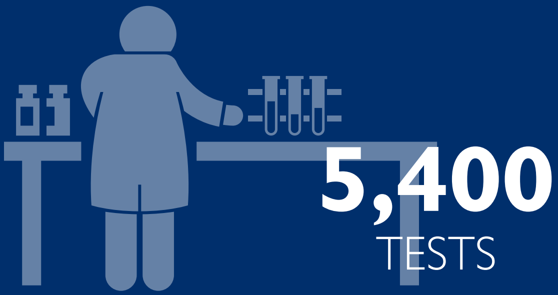
Global Health Security Agenda

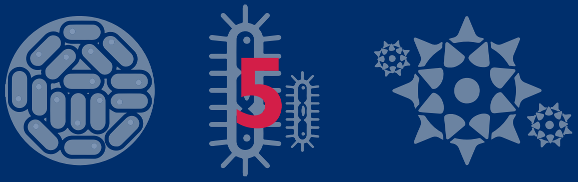
DEVELOPED the One Health Workforce by training more than 70 people in Mongolia.

OPERATIONALIZED One Health surveillance and sampled over 3.2K animals, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- State Central Veterinary Laboratory





DETECTED 5 unique subtypes of Influenza A virus

ARIUNBAATAR BARKHASBAATAR

Avian Specialist, *Wildlife Conservation Society*

"In 2016 I started working as an avian specialist on the PREDICT project while also working to complete my master's degree on raptor populations in Mongolia. I led the spring and fall field sampling events for three regions in Mongolia and trained PREDICT partners in bird identification, survey methods, and safe handling of avian species. The surveys included shoreline transects, examinations of dead and sick birds, conducting post-mortem examinations, and collection of guano samples. I acquired a lot of field experience and wildlife disease knowledge from the PREDICT project, especially bird necropsy and sample collection."

ENKHTUVSHIN SHIILEGDAMBA

Country Coordinator, *Wildlife Conservation Society*

"I am the Wildlife Conservation Society Mongolia Program director for the last 6 years and leading environmental and conservation focused projects as well as livestock, wildlife and human health interface disease projects such as the PREDICT project. I am very proud to be part of the global PREDICT project and to be able to contribute some of my knowledge and capacity support not only to the project but also to the Mongolian professional organizations that partnered with us to build their capacity and knowledge and to support One Health."

BATCHULUUN DAMDINJAV

Laboratory Testing & Diagnostic Liaison, *Wildlife Conservation Society*

"During the project, we organized an online training on gene sequencing and data analysis for SCVL employees with PREDICT experts. It was a great training opportunity and training video materials were provided for SCVL employees. The barcoding tests were done on all positive samples and on some targeted negative samples by Cytochrome B and COI specific PCR. Through the project, I learned the importance of sampling methods for field surveillance and genetic analysis, barcode testing, data cleaning, and data analysis."

ACHIEVEMENTS

- One Health teams including specialists from the provincial zoonotic disease centers, veterinary units, and environmental agencies were trained to perform field sampling and data collection for avian influenza surveillance following field protocols.
- Samples from the field were successfully stored and transported with cold chain to the State Central Veterinary Laboratory where influenza virus detection and subtyping of influenza A positive samples was completed.
- One Health teams were successfully prepared and trained to quickly and efficiently respond to wildlife disease outbreaks in remote parts of Mongolia including Central, Eastern, and Western regions. Since 2016 they supported responses to peste des petits ruminants (PPR) disease outbreaks in wildlife and investigated a large-scale die-off of water birds.
- One Health cross-sectoral information sharing was strengthened by distributing test results and data on risk characterization from the project to all the national agencies that collaborate on zoonotic disease policy and surveillance in Mongolia including GAVS, NCZD, NIC, and SCVL.
- Investments in avian influenza field surveys and laboratory capacity building in Mongolia resulted in 66 sampling events at 26 sites between May 2016 and October 2018 and the collection of 3,243 samples that were tested for avian influenza virus. The surveys and collection of guano samples were focused on waterfowl, including swans, ducks, geese, and shelducks, and brought laboratory scientists out in the field to improve their field expertise.
- Our work made a significant contribution in monitoring critical habitat for globally important populations of wild birds and raised interest among veterinary, human health, and environmental agency specialists alike on monitoring and conservation of wild avian species.

ONE HEALTH SURVEILLANCE

PHOTO 1. ***Necropsy exam of a common shelduck at Sangiin Dalai Lake, Western Mongolia in August 2018.***

PHOTO: PREDICT/MONGOLIA

In Mongolia, sampling for avian influenza surveillance was performed at 26 sites (Figure 1) collecting 3,243 bird guano and tissue samples. The team also collected opportunistic swab samples from sick birds unable to fly, and performed necropsy examinations on dead birds that were encountered during field activities (Photo 1). Guano samples were collected from wild water birds including swans (*Cygnus sp.*), ducks (*Anas sp.*), geese (*Anser sp.*) and shelducks (*Tadorna sp.*). Opportunistic sampling was performed including oral swabs from 19 Cinereous vultures (*Aegypius monachus*), tissue samples from eight arctic herring gulls (*Larus smithsonianus*) and four common shelducks (*Tadorna tadorna*), and tissue or swab samples from one to two individuals of another seven species.

All samples were safely screened for influenza virus using PREDICT protocols at the State Central Veterinary Laboratory (SCVL) and positives were confirmed by cloning and sequencing of the PCR products. SCVL also performed barcoding to assist with host identification.

Testing of bird guano samples found 57 (1.8%) samples positive for influenza A and were further subtyped at the SCVL. Although none of the subtypes detected were highly pathogenic avian influenza, the findings of low pathogenic avian influenza viruses are important for understanding what is circulating in wild birds. The proportion of positives was significantly higher in the West (1.7%, n=874) and Central regions (3.0%, n=1371) compared to the Eastern region (0.1%, n=947). The H5N1 outbreaks previously reported in Mongolia were detected mostly in the Central and Western regions repeatedly in 2005, 2006 and 2009; while H5N1 was detected in the Eastern Region only in 2010. As with the number of positives, the number of subtypes detected was also higher in the Central and Western region as five subtypes detected, H2N2, H3N8, H4N6, H6N2, and H10N7; compared to only one subtype, H2N2, in the Eastern region. The proportion of positives also varied between sampling years, from a high of 3.9% in 2016, to 0.6% and 0.5% in 2017 and 2018 respectively. Within years, the fall season, including the months of August, September, and October, tended to have more positives (2.3%) than the spring season (1.2%), which included the months of April, May, and June. Finally, the highest proportion of positives by host were in shelducks (genus *Tadorna*, 2.9%), followed by herring gulls (genus *Larus*, 2.0%) and ducks (genus *Anas*, 1.8%).

VIRUS TABLE (2015-2019)

SAMPLING LOCATION (PROVINCE)	VIRUS	SPECIES	# OF POSITIVE INDIVIDUALS TOTAL	SPRING SEASON	FALL SEASON
Arkhangai	Influenza A, subtype H10N7	Unidentified Anas Duck	1	0	1
	Influenza A, subtype H3N8	Ruddy Shelduck, Unidentified Anas Duck, Whooper Swan	19	12	7
Bulgan	Influenza A, subtype H3N8	Ruddy Shelduck, Unidentified Anas Duck	10	0	10
	Influenza A, subtype H4N6	Unidentified Anas Duck	1	1	0
Khovd	Influenza A, subtype H6N2	Unidentified Anser Goose	3	3	0
Khuvsgul	Influenza A, subtype H3N8	Ruddy Shelduck	2	0	2
	Influenza A, subtype H4N6	Ruddy Shelduck	8	0	8
Sukhbaatar	Influenza A, subtype H2N2	Ruddy Shelduck	1	1	0
Uvs	Influenza A, subtype H2N2	Unidentified Larus Gull	1	1	0
	Influenza A, subtype H4N6	Unidentified Anas Duck	4	0	4
Zavkhan	Influenza A, subtype H10N7	Ruddy Shelduck	7	0	7
Total			**57**	**18**	**39**

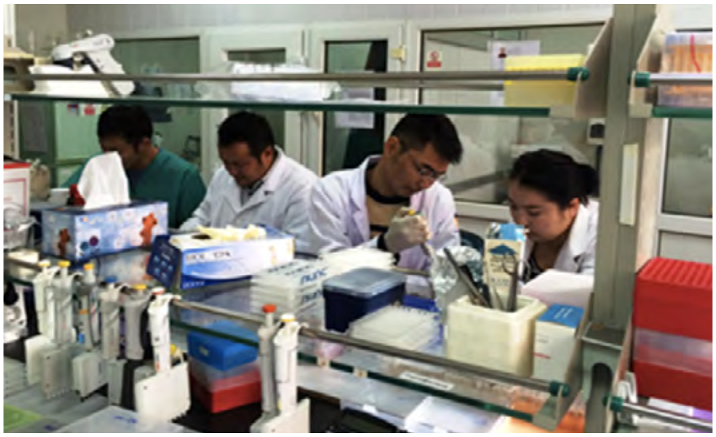


PHOTO 2. ***Laboratory screening of samples at SCVL.***

PHOTO: PREDICT/MONGOLIA

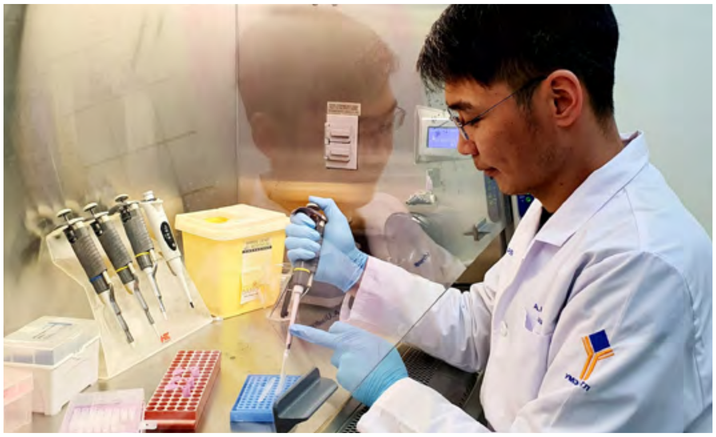


PHOTO 3. ***Laboratory testing for influenza virus was conducted at SCVL using PREDICT protocols.***

PHOTO: ULAANKHUU ANKHANBAATAR

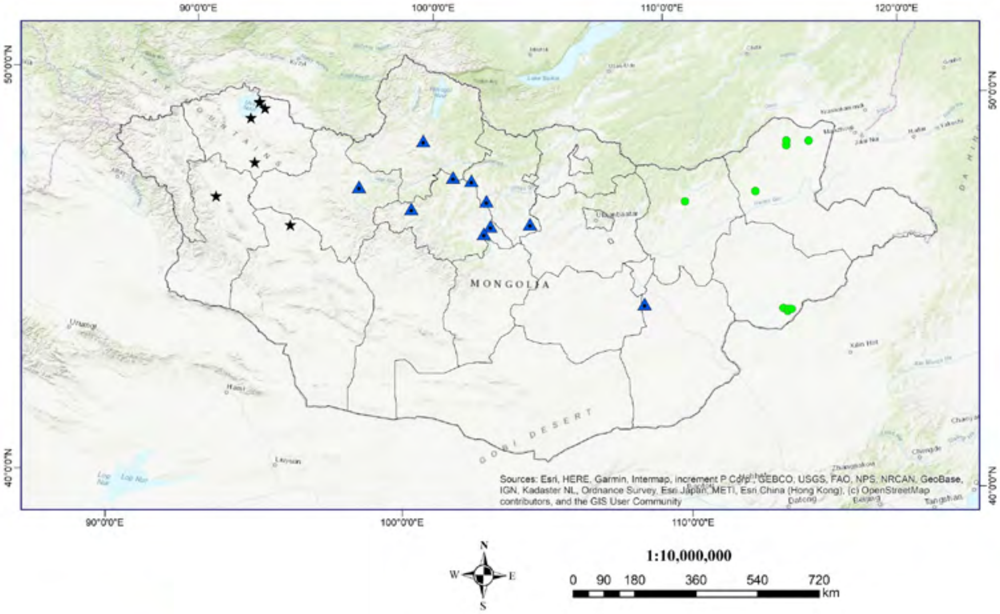


FIGURE 1. ***PREDICT surveillance sites in Mongolia, black stars, blue triangles, and green circles representing the Western, Central, and Eastern region sites respectively.***

OUTBREAK PREPAREDNESS & RESPONSE

TABLE 2. DEAD & SICK BIRDS LIST AT SANGIIN DALAI LAKE, 25-27 AUGUST, 2018

SPECIES SCIENTIFIC NAME	SPECIES COMMON NAME	DEAD	SICK	TOTAL
Tadorna tadorna	Common Shelduck	121	49	170
Tdorna ferruginea	Ruddy Shelduck	102	27	129
Chroicocephalus ridibundus	Black-headed Gull	12	1	13
Recurvirostra avosetta	Pied Avocet	1	4	5
Larus mongolicus	Mongolian Gull	1	2	3
Anas platyrhynchos	Mallard	2		2
Anas acuta	Northern Pintail	1	1	2
Himantopus himantopus	Black-winged Stilt	1		1
Sterna hirundo	Common Tern	1		1
Calidris minuta	Little Stint		1	1
Aythya fuligula	Tufted Duck		1	1
Calidris temminckii	Temminck's Stint		1	1
Unknown species		12		12
Total		254	87	341

PHOTO 4. ***Mass die-off of wild birds around Sangiin Dalai Lake, Gobi-Altai Province***

PHOTO: PREDICT/MONGOLIA

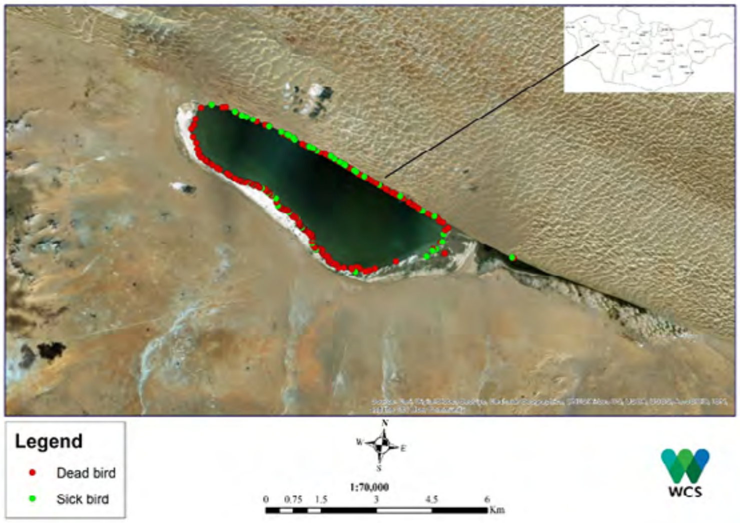


FIGURE 2. ***Map of dead and sick birds at Sangiin Dalai Lake, August 25-27, 2018.***

The PREDICT project worked with local partners to improve wild bird surveillance and to support early detection and rapid response to avian influenza outbreaks. This preparation paid off in August 2018 when a mass mortality event of wild birds of unknown cause occurred at Sangiin Dalai Lake, located in the far western region. The Sangiin Dalai Lake (47.40°N, 94.96°E) is a high saltwater lake located at the front edge of the Mongol Els sand dune in Khokhmorit Soum, Gobi-Altai Province, and is surrounded by sand dunes and small sandy hills.

In response to an emergency call from local partners the team performed sampling around the lake and met with local partners in the veterinary and environmental sectors on 25-27 August, 2018. Surveys included collection of guano samples and a 15km shoreline transect to count sick and dead birds. A total of 341 dead and sick birds, including 254 dead and 87 sick birds belonging to 12 species, were counted. Most were common shelducks (n =170) and Ruddy shelducks (n = 129) (Table 2). A total of 85 samples were collected including 25 tissue samples, five oral swabs, five cloacal swabs from dead birds and 50 guano samples from geese.

Although avian influenza was later ruled out, the event demonstrated a strong One Health outbreak response involving key stakeholders. The cause of this die-off remains unknown. Testing found that animals were negative for avian influenza and Newcastle disease virus, an avian paramyxovirus. In-country laboratory and field teams determined that avian botulism was the most likely cause of the large-scale die-off given the negative virus test results and absence of gross or histological lesions in birds. Botulism testing was not performed to confirm suspicions due to limited capacity and resources.

SPECIAL FEATURE

INTEGRATING WILDLIFE CONSIDERATIONS INTO PESTE DES PETITS RUMINANTS CONTROL

In early January 2017, environmental rangers were encountering an unprecedented number of sick and dead saiga antelope (*Saiga tatarica mongolica*) – a critically endangered species – as they drove their survey transects. It was quickly determined that the disease killing the saiga was peste des petits ruminants (PPR), a paramyxovirus that had first been confirmed in Mongolian sheep and goats in 2016. The outbreak of PPR in Mongolian saiga represents the first time the disease was recorded in a free-ranging antelope species and took both the wildlife conservation and livestock health experts by surprise.

Learn more here: **bit.ly/OH-study-Mongolia**

PREDICT
MYANMAR

ONE HEALTH IN ACTION (2014-2020)

Creating the first major framework for the incorporation of environmental and wild animal health at the forefront of infectious disease emergence, and implementing One Health across Myanmar's community, university, and government sectors.

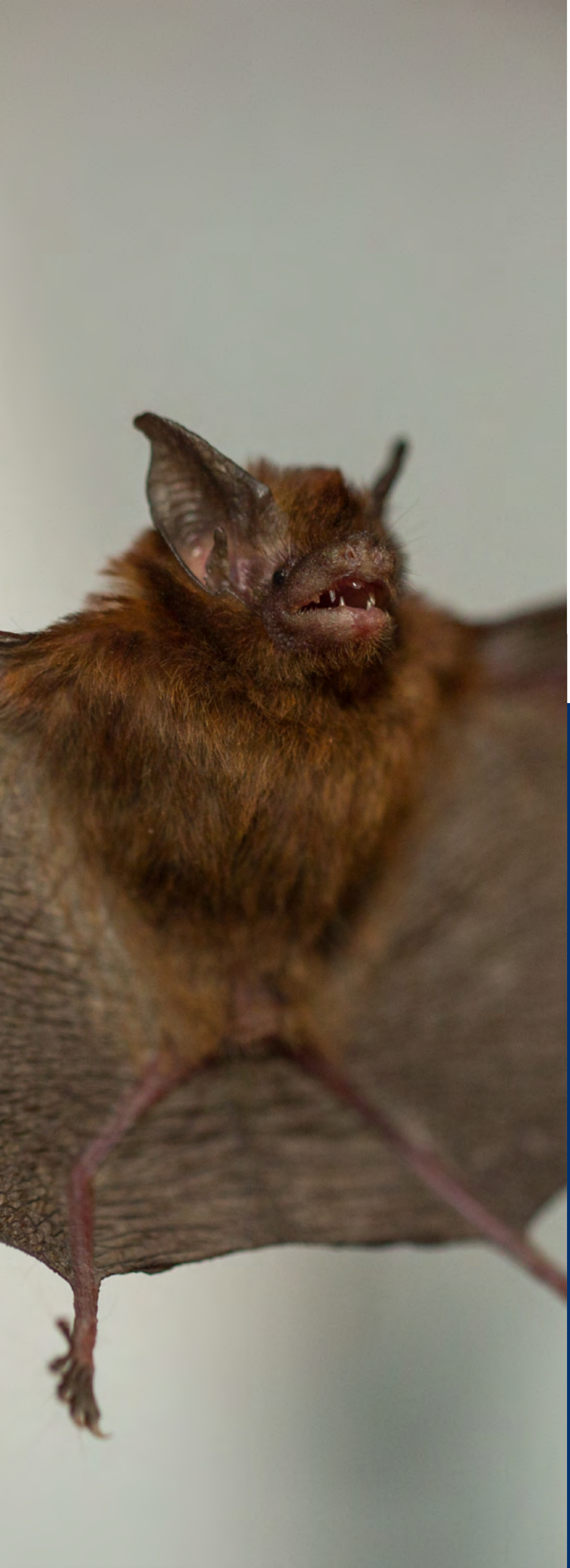
At the crossroads between South and Southeast Asia paired with its developing status as a nation, Myanmar is a hotspot for zoonotic disease emergence. Multiple avian influenza outbreaks since 2009 have underscored this concern, which has prompted the creation of a national One Health program involving members from the Ministry of Health and Sports (MOHS) and the Ministry of Agriculture, Livestock, and Irrigation (MOALI).

Recent democratization, rapid development and commercialization have led to significant land-use change and an increased demand for alternative resources such as wildlife and their products. Owing to the country's unique geography with limited access, partial self-governance at the northern borders, and an enforcement infrastructure in its infancy, the wildlife-human interface remains under-evaluated and a source of concern for zoonotic disease emergence.

PREDICT created the first major One Health surveillance framework in Myanmar. Through coordinated surveillance of people and wildlife at high-risk disease transmission interfaces, PREDICT worked across Myanmar's community, university, and government sectors to strengthen mechanisms to detect and respond to zoonotic disease threats and build capacity in both the field and laboratory for the next generation of Myanmar's One Health workforce.

LOCAL PARTNERS

- Department of Medical Research (DMR), Ministry of Health and Sports
- Livestock Breeding and Veterinary Department (LBVD), Ministry of Agriculture, Livestock and Irrigation
- Forestry Department of Ministry of Natural Resources and Environmental Conservation



>480

DEVELOPED the One Health Workforce by training more than 480 people in Myanmar.

>3.6K

OPERATIONALIZED One Health surveillance and sampled over 3.6K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Livestock Breeding & Veterinary Department
- Department of Medical Research

12,084
TESTS

9 PREDICT

1 KNOWN

DETECTED 10 unique viruses in both animal and human populations.

OHNMAR AUNG

Country Coordinator & Medical Surveillance Officer, *Smithsonian Conservation Biology Institute's Global Health Program*

"After almost 20 years of experience as a public health and health social scientist, this is the first time I have seen the conceptualization of public health issues related to wildlife, livestock and human interfaces in Myanmar. As a doctor and public health professional, it has been an incredible experience to partake in studying communicable disease transmissions and virus outbreaks while understanding it from the animal and environmental health perspective. It was a great opportunity working for PREDICT, obtaining a comprehensive scope of the One Health Concept and studying zoonotic diseases at their source."

KYAW YAN NAING TUN

Veterinary Field Surveillance Officer, *Smithsonian Conservation Biology Institute's Global Health Program*

"As a veterinarian in Myanmar, I became very knowledgeable about wildlife health and potential disease transmission among wildlife, livestock, and humans from the experiences working for PREDICT. I have limited expertise about wildlife health and wildlife medicine in our University of Veterinary Science. I can apply these experiences and knowledge of PREDICT implementations in my future career while also providing education to the community and my junior colleagues."

ACHIEVEMENTS

- PREDICT conducted the first major study incorporating concurrent wildlife, human, and livestock viral disease surveillance activities in Myanmar.
- 150 trainees (50 males, 100 females) in over 30 training sessions conducted in all relevant One Health surveillance and diagnostic elements for a strong local One Health capacity.
- Detected six novel coronaviruses in bats and one novel paramyxovirus in a rodent at high-risk disease transmission interfaces including bat caves used for ecotourism, guano collection and religious ceremonies.
- Advised on the National One Health Strategic Plan resulting in the inclusion of zoonotic diseases originating in wildlife as a primary focus.
- Through PREDICT project guidance, the Ministry of Natural Resources and Environmental Conservation was invited to participate in designing of the National One Health Strategic Plan, which provided invaluable environmental context for the first time in planning for public and animal health.
- With local partners, DMR and LBVD, the PREDICT team convened a symposium on "One World One Health – PREDICT Myanmar" during the 47th Myanmar Health Research Congress in 2019. This marked the first time the congress set aside a section to offer a major focus on health related to wildlife and the environment for over 150 participants in the health field.
- Adapted the Smithsonian's National Museum of Natural History's mobile "Outbreak!" DIY exhibition materials to Myanmar specific content and used it during ministry and community engagement meetings as well as at the US Embassy to communicate the success of the PREDICT project and share strategies for public health interventions and disease prevention. Multiple exhibitions have been printed and shared for long-term display at the three ministry headquarters (MOHS, MOALI, MONREC) in NayPyiTaw as well as the University of Medicine and University of Veterinary Sciences.

ONE HEALTH SURVEILLANCE

Strategy for nationally-recognized priority zoonotic diseases

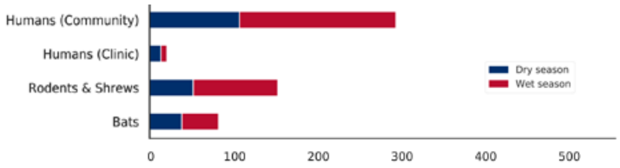
PREDICT conducted One Health surveillance to detect priority zoonoses and emerging diseases in wildlife and people at high-risk animal-human interfaces. At the same time, PREDICT conducted interviews with local community members to better understand the social and behavioral factors associated with zoonotic disease transmission. By facilitating dialogue and sharing information between interdisciplinary partners, PREDICT strengthened One Health platforms and knowledge exchange between animal and public health sectors.

To enable assessment of potential trends in viral spillover and spread across space, time, and season, the PREDICT team conducted surveillance activities at three key sites in Hpa-An in Kayin State, North Yangon in Yangon Region, and Shwebo in Sagaing Region over a three-year period from May 2016 to September 2018. These sites were identified by stakeholders as locations of national importance due to their dynamic wildlife-livestock-human interfaces and potential zoonotic disease exposure.

During ten seasonal surveillance trips over two years, the PREDICT team collected over 20,000 specimens from bats, rodents and people at previously investigated interfaces, where there was a high level of interaction between animals and humans.

Laboratory testing of samples was conducted in PREDICT partner laboratories at the Ministry of Health and Sports (MOHS) Department of Medical Research for human samples, and the Ministry of Natural Resources and Environmental Conservation (MOALI) Livestock Breeding and Veterinary Department for animal samples. Both laboratories used standardized PREDICT protocols to screen for both priority zoonotic viruses of national public health concern, along with other novel and emerging viral threats.

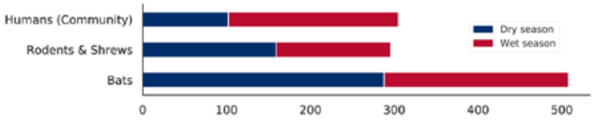
HPA-AN



Numbers of individuals sampled by taxa group in Hpa-An

A rural agricultural community characterized by crop production and free-roaming livestock, Hpa-An features extensive, sacred bat caves that are used by people for guano harvesting, religious pilgrimages, and ecotourism. These caves house roosting colonies of approximately 300,000-500,000 bats during high season. Additionally, one cave complex maintains a resident colony of 200 macaques that regularly and directly interact with visitors through hand-feeding and physical contact. Each cave hosts between 100-500 local and international tourists daily. Traditional and religious practices of these communities include wildlife consumption and removal of footwear to visit sacred caves, respectively. Concurrent surveillance of bats, rodents, and macaques was conducted at the cave interface, alongside behavioral risk assessments and sampling of at-risk residents living near and using the bat caves for religious and livelihood purposes.

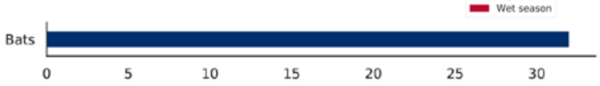
NORTH YANGON



Numbers of individuals sampled by taxa group in North Yangon

Occupying 1,540 acres, Hlawga National Park is home to more than 2,000 free-roaming macaques (*Macaca mulatta* and *M. nemestrina*), which interact with ecotourists. Other wildlife in the park include bats, deer, boar, gaur, birds, and rodents, while domestic species including cattle, chickens, ducks, and dogs range nearby. The park receives between 200-1,000 tourists and visitors every day. PREDICT's One Health surveillance strategy targeted bats and rodents in and surrounding the National Park, as well as residents of nearby villages that may be exposed to wildlife through their work in animal and crop production, and hunting. The PREDICT team also conducted syndromic surveillance of patients at Hmawbi Hospital which provides basic health services to the local community, most of whom live in and around the Hlawga National Park.

SHWEBO



Numbers of individuals sampled by taxa group in Shwebo

Shwe Zay Ti Monastary Compound is a rural 4,300 square meter area located between Shwebo and Yangon in central Myanmar. It is a large monastery with about 100 monks and locals residing in close contact with wildlife like rodents and bats as well as farm-raised domestic animals. Surveillance targeted bat guano after the PREDICT team identified several high-risk interfaces with people in the region.

HUMAN SURVEILLANCE

DEMOGRAPHICS OF PARTICIPANTS ENROLLED IN NORTH YANGON & HPA-AN

	CLINIC-BASED SURVEILLANCE	COMMUNITY-BASED SURVEILLANCE			
DISTRICT	North Yangon				Hpa-An
SITE ENROLLED	Hmawbi Hospital (n=20)	Hlawga National Park (n=38)	Hmawbi villages (n=332)	Oakkan (n=12)	Hpa-An villages (n=306)
GENDER					
MALE	11 (55%)	22 (58%)	179 (54%)	3 (25%)	139 (45%)
FEMALE	9 (45%)	16 (42%)	153 (46%)	9 (75%)	167 (55%)
AGE GROUP					
CHILD (<18)	10 (50%)	0 (0%)	25 (8%)	2 (17%)	27 (9%)
ADULT	10 (50%)	38 (100%)	307 (92%)	10 (83%)	279 (91%)
AGE					
MEDIAN [MIN, MAX]	18 [3,62]	42 [18,67]	37 [2,79]	29 [3,54]	45 [2,77]

Through the PREDICT project's multidisciplinary One Health surveillance approach, our team worked with local communities to better understand social and behavioral factors associated with risks of zoonotic disease transmission, focusing on the "how" and "why" of risk. Using data-driven methods, the PREDICT team identified potential policies and intervention strategies that might be effective in preventing or mitigating zoonotic viral spillover and spread. Insights into behavior help direct efforts to raise public awareness of personal and occupational zoonotic disease risks.

Between July 2017 and August 2018, 688 people from the community and 20 patients at the hospital completed questionnaires about their behavior and livelihoods at high risk animal-human interfaces and provided samples for virus testing. Participants were community members from townships in and surrounding two regions: Hmawbi, near Hlawga National Park in North Yangon, and Hpa-An. Of the respondents, 50% were female, and 50% male. The average age of participants was 40 years old, ranging from 2 to 79 years old. The majority (67%) were long-term residents, living in their community for over ten years.

EPIDEMIOLOGICAL & BEHAVIORAL RISK

Among 708 interviewed respondents, 82% were either directly exposed to wildlife and livestock through their livelihoods, or indirectly exposed by using animal products for crop production. Primary practices leading to exposure to wildlife and their byproducts included rodent and bat consumption and guano harvesting.

SANITATION IN THE COMMUNITY

Basic food, water, and sanitation hygiene practices are commonplace within the households in all communities. Preventative measures such as treating water, covering food, and using dedicated areas for human waste are highly prevalent. However, nearly half the people have observed animal feces, or animal bite marks in their food in the last year, suggesting the current measures being used are insufficient, thus presenting a disease transmission risk. Awareness of disease risks associated with open wounds while butchering was lacking, despite reporting appropriate wound care in daily life.

PERCEPTION OF DISEASE RISK FROM ANIMALS

Knowledge of the risks of disease transmission associated with animal contact were low within the communities. Over 98% of surveyed respondents reported being exposed to live animals (small scale and household livestock in most of their residences) through daily practices, working, and living conditions, including handling, raising, petting, and sharing of dwellings.

Among 708 interviewees, 67% reported experiencing some kind of illness within the previous year. Of these, the majority had fever associated with influenza-like illness, severe acute respiratory illness, or flu-like symptoms. Most respondents were unable to link their clinical signs to possible etiologies, and only a third speculated that the source of transmission was from animals or sick people. However, people who were exposed to live and dead animals through their livelihoods, households, and consumption practices were more likely to have experienced fever symptoms in the previous year.

Interestingly, 96% of people did not report contact with bats despite close proximity and observations of people entering bat caves at both sites. Field observations suggest contact is likely more prevalent than declared and possibly because the question was misunderstood. Of the 31 people who reported contact, bats living in houses and fecal contamination of food were the most common routes. Rodents were most frequently found in the home, with some people hunting, slaughtering, or consuming them. Of concern, people were keeping bats, primates, and rodents as pets, an activity with very intimate interactions and high-risk for disease transmission.

Interactions with non-human primates were largely limited to workers in a protected area or zoo/sanctuary, with handling and or being scratched/bitten being the most frequent type of interaction reported. People that were scratched by animals were twice as likely to have reported falling ill in the last year. Conversely, respondents who practiced good hygiene following animal-related scratches and bites were 60% less likely to have fallen ill in the previous year, thus suggesting that practicing good hygiene protects against contracting disease.

High-risk behaviors such as consumption of raw meat and sick animals was commonly reported. Our survey identified that the respondents who consumed animals (when found deceased) were more likely to report an illness in the previous year. Nearly all respondents had direct contact with poultry/fowl, which are also the most common taxa to be eaten raw or when sick. These intimate and risky behaviors may facilitate transmission of viruses such as avian-origin influenza into people, especially during a disease outbreak.

OCCUPATIONAL DISEASE RISKS

The agricultural sector consisting of agricultural, livestock, fisheries, and forestry industries comprised more than 29.8% of Myanmar's 2014-2015 gross domestic product and served as the primary source of livelihood for 61.2% of the population (Aung et al., In progress). The communities we surveyed reflected this demographic with over 50% of people working in crop or animal production. Despite reported concerns about livestock health due to their importance for food and financial security, we found a generally poor awareness of disease association between human and animal health.

At one site, a local family has been collecting guano for commercial purposes from a cave system for over three generations. Practices for collection are similar to other areas around the region with little to no skin or respiratory protection nor any standards for washing after handling guano. Because the collection site is also a religious temple, family members are required to remove all footwear prior to entering the cave. All participants in the routine guano extraction often take refuge in the cave, eating lunch and taking short naps. Reports of poor health following guano collection is common but transient and medical attention is rarely sought after.

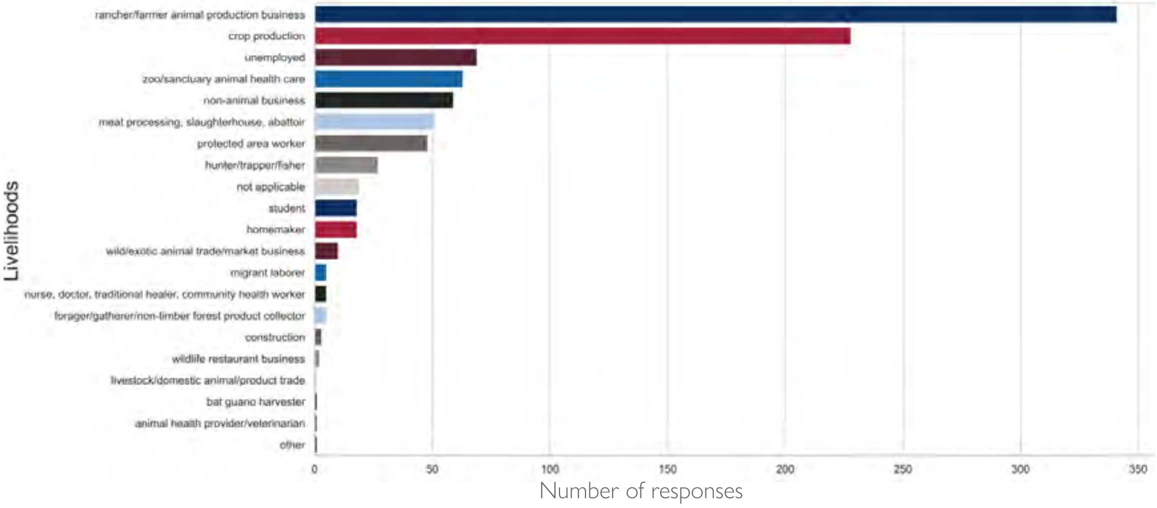


FIGURE 1. Livelihoods of participants from townships in and surrounding two regions: Hmawbi, near Hlawga National Park in North Yangon, and Hpa-An

VIRUS FINDINGS IN PEOPLE

PREDICT partner laboratory MOHS's Department of Medical Research safely tested oral swabs from 607 people, including 20 from Hmawbi Hospital in North Yangon, and 587 from members of the community (305 Hpa-An, 280 North Yangon). A total of 3,642 individual tests were conducted to detect viruses within priority virus groups (coronaviruses, filoviruses, paramyxoviruses, and influenza viruses) using consensus PCR. Coronavirus, filovirus, and paramyxovirus results were negative, suggesting no one had an active infection for viruses in these families.

Influenza A virus was detected in 16 oral samples from participants at two study sites; 11 in Hpa-An and five in North Yangon. One person was a male patient at the Hmawbi Hospital, while the rest were apparently healthy community members, comprising 10 females and five males. Age of positive participants ranged from 5 to 63 years old with a median age of 41. Influenza A virus is known to infect birds and mammals and is a common cause of flu like symptoms in people, including fever, cough, sore throat, muscle aches, conjunctivitis, and in severe cases pneumonia. Severity of infection is dependent on the virus subtype and immune response of the person infected. Most people (12/16) infected with Influenza A virus at the time of PREDICT surveillance reported having a fever in the previous year, of which eight had an influenza-like illness. Influenza A virus was detected in both the wet and dry seasons, and all those who tested positive had had direct contact with animals for their livelihoods, especially for females who reported direct exposure to animals and their products for cooking, meat processing and raising animals at home. Further investigation is required to subtype the influenza A viruses detected, which would give insight into the source of the virus.

WILDLIFE SURVEILLANCE

PREDICT tested 1,305 samples from 907 individual animals (443 rodents, 464 bats), and 155 bat guano samples from high risk animal-human disease transmission interfaces in North Yangon, Hpa-An and Shwebo. Samples collected from macaques are to be tested at a later date. Specifically, our PREDICT partner laboratory at MOALI's Livestock Breeding and Veterinary Department (LBVD) tested 8,442 rectal swabs, oral swabs, and guano samples for priority virus groups (coronaviruses, filoviruses, paramyxoviruses, flaviviruses, and influenza viruses) using consensus PCR which tests for active viral infection at the time of capture and sampling.

ANIMALS TESTED FOR PRIORITY VIRUS GROUPS AT HIGH-RISK DISEASE TRANSMISSION INTERFACES IN NORTH YANGON & HPA-AN

DISTRICT	NORTH YANGON				HPA-AN			SHWEBO	
SITE	Hlawga National Park	Hlawga Village	Hmawbi Village	Oakkan	Cave 1	Cave 2	Hpa-An Village	Monastery Compound	TOTAL
TAXA (no. positive/ tested)									
BATS	3/47	1/3	0/1	0/28	3/116	0/252	0/17	-	7/464
BAT GUANO	-	-	-	-	36/86	4/73	-	0/32	40/191
RODENTS	0/111	0/36	-	-	0/2	1/291	0/3	-	1/443

VIRUS FINDINGS IN WILDLIFE

Novel coronaviruses and paramyxoviruses were detected in bats, bat guano and rodent samples in caves near communities in Hpa-An, and in bats at Hlawga National Park in North Yangon (Table 2). Viruses were detected in animals in both the wet and the dry seasons. There is no evidence at this time to suggest that these novel viruses pose a threat to human health. These findings prompted a direct response from the LBVD Director General, Dr. Ye Tun Win:

"The identification of new viruses from bats and rats in Myanmar is due to the efforts of the PREDICT project and addressed the need for long-term collaboration across the three ministries (the MOHS, MOALI and MONREC) in strengthening the capacity for disease surveillance and laboratory testing to identify new pathogens which could potentially be transmitted between animals and humans."

BAT GUANO

Four novel coronaviruses (PREDICT_CoV-47, PREDICT_CoV-82, PREDICT_CoV-92, PREDICT_CoV-96) and one paramyxovirus (PREDICT_PMV-48) were found in bat guano from caves in Hpa-An.

The types of virus found differed by site; PREDICT_CoV-92, PREDICT_CoV-96 and PREDICT_PMV-48 were detected in guano samples from roundleaf bat colonies (*Hipposideros* species) in Hpa-An Cave 1, while PREDICT_CoV-82 and PREDICT_CoV-47 were found in samples from wrinkle-lipped free-tailed bat colonies (*Chaerephon plicatus*) in Hpa-An Cave 2. Several samples were positive for more than one virus which is not surprising given that the samples are pooled fecal/urine (guano) from many individuals and a bat can be infected with more than one virus. All bat guano samples were negative for additional priority virus groups (filoviruses, flaviviruses, and influenza viruses).

BATS

Only 2% (7/464) of bats captured and sampled tested positive for viruses, all of which were novel coronaviruses. The majority of positive samples were from rectal swabs, with only one oral swab testing positive for PREDICT_CoV-96.

All bat samples were negative for the other priority virus groups (filoviruses, paramyxoviruses, flaviviruses, and influenza viruses).

Coronaviruses were detected in bats sampled at both Hpa-An and North Yangon. Three roundleaf bats (*Hipposideros* species) in Hpa-An Cave 1 were positive for two novel coronaviruses PREDICT_CoV-93 and PREDICT_CoV-96, the latter having also been detected in bat guano collected from the same cave. Three greater Asian house bats (*Scotophilus heathii*) bats in Hlawga National Park, North Yangon, were positive for the novel PREDICT_CoV-35, one of which also tested positive for novel PREDICT_CoV-90. Interestingly, PREDICT_CoV-35 was also detected in an unidentified tomb bat (*Taphozous* sp.) in Hlawga Village which is adjacent to Hlawga National Park.

RODENTS

The majority (84%) of rodents were rats belonging to the *Rattus* genus (*Rattus exulans, Rattus norvegicus, Rattus rattus, Rattus tanezumi*), while the lesser bandicoot rat (*Bandicota bengalensis*), the Chinese white bellied rat (*Niviventer confucianus*) and Asian house shrews (*Suncus murinus*) were also sampled. Out of 443 animals tested, one Oriental house rat (*Rattus tanezumi*) in Hpa-An Cave 2 tested positive for a novel paramyxovirus PREDICT_PMV-132 in both its rectal and oral swabs. All rodents were negative for other priority virus groups (coronaviruses, filoviruses, flaviviruses, and influenza viruses).

VIRUS TABLE (2015-2019)

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	PREDICT_CoV-35	Greater Asian House Bat, Unidentified Taphozous Bat	Hmawbi	4	3	1
	PREDICT_CoV-47	Wrinkle-Lipped Free-Tailed Bat	Hpa-An	1	1	0
	PREDICT_CoV-82	Wrinkle-Lipped Free-Tailed Bat	Hpa-An	3	3	0
	PREDICT_CoV-90	Greater Asian House Bat	Hmawbi	1	1	0
	PREDICT_CoV-92	Intermediate/Grand Roundleaf Bat Species Complex	Hpa-An	36	0	36
	PREDICT_CoV-93	Intermediate/Grand Roundleaf Bat Species Complex	Hpa-An	3	0	3
	PREDICT_CoV-96	Intermediate/Grand Roundleaf Bat Species Complex	Hpa-An	4	0	4
Paramyxovirus	PREDICT_PMV-48	Intermediate/Grand Roundleaf Bat Species Complex	Hpa-An	4	0	4
	PREDICT_PMV-132	Oriental House Rat	Hpa-An	1	0	1
Influenza virus	Influenza A	Human	Hpa-An, Hmawbi, Hmawbi Hospital	16	9	7
Total				**73**	**17**	**56**

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

The PREDICT team was committed to community engagement and worked with local villages and partners to raise awareness of zoonotic disease threats and strengthen capacity for prevention, surveillance, and detection. Sensitization meetings were held in all PREDICT project sites over the life of the project. At these meetings, our team worked with local communities to create a dialogue and build trust for implementation of surveillance activities and a platform to communicate One Health approaches.

Implementation of outreach and engagement activities provided a forum for PREDICT to empower community members to make the most informed decisions regarding their own health security on an individual level and from a community perspective. Direct community engagement in our concurrent surveillance sites of Hpa-An and North Yangon involved project introduction, permission requests, progress reporting, and involvement in surveillance activities. In 2017, the PREDICT team organized a One Health day event at Hlawga National Park that was very well attended, offering an opportunity to further engage with the community at large.

Through behavioral risk characterization, PREDICT was able to identify risk factors and to target zoonotic disease risk mitigation at the local scale. In community meetings, the PREDICT team provided summaries of project findings and shared resources to raise awareness of disease. Key visual education tools were provided to support specific public health recommendations, including a mobile "Outbreak" DIY panel exhibition developed in conjunction with Smithsonian's National Museum of Natural History, and PREDICT's behavior change picture book *Living Safely with Bats* (available at **www.predict.global**) that is designed to minimize risks of infection to bat-borne viruses such as Ebola, Marburg, and MERS.

STRENGTHENING CAPACITY

The PREDICT team targeted sustainable, lasting health impacts through training, education, outreach and capacity building with in-country partner institutions. The team trained 150 individuals in the One Health field, provided professional development for over 400 students, and educated and empowered local communities to make informed decisions that benefit community health. PREDICT strengthened and developed capacity through hands-on skill training, didactic lectures, development of supply chains, and laboratory equipment procurement, and distribution of educational resources at the community, university, and government levels. Cross-training and updates were also provided to relevant international counterparts including FAO, WHO, and CDC representatives for further collaboration.

The PREDICT team has been regarded by the ministries as an integral partner in developing plans for zoonotic infectious disease control and response, and as such PREDICT actively participated in the development of Myanmar's inclusive One Health National Strategic Plan (2017-2022) in close collaboration with relevant government departments, three universities, and international partners (WHO, FAO, CDC). This activity effectively bridged the gap between advocacy and action related to wildlife and environmental health policy. The PREDICT project played key facilitation and coordination roles in bringing representatives together from three different ministries for coordinated One Health planning and activities. Specifically, the PREDICT team involved representatives from the three relevant ministries for animal, human and environmental health in all activities and provided training on zoonotic disease surveillance. Through the PREDICT project's activities in Myanmar, representatives from the Ministry of Natural Resources and Environmental Conservation (MONREC) were invited to participate in national One Health implementation meetings, marking the first time MONREC began participating in the protection of human health.

Community health workers and forestry department officials sought to improve their identification skills of high-risk interfaces and assisted in the selection of sites for PREDICT project activities. By training these key workers, they are now better equipped to identify and address potential sources of zoonotic disease exposure and behavioral risk factors in the community. Their newfound knowledge is being disseminated amongst the community members and is being passed on to neighboring community health workers, creating a network of trained One Health professionals, especially with regard to disease investigation and treatment therapy for hospitalized patients.

Beyond the direct community involvement, PREDICT established relationships to develop the higher education curriculum for the next generation of One Health professionals by offering lectures to three major universities including the University of Medicine, the University of Veterinary Sciences, and the University of Yangon's Zoology Department. Additionally, cross-training presentations on the PREDICT One Health strategy were offered to the scientific and physician communities through the Department of Medical Research.

Additionally, the PREDICT project enhanced Myanmar's national laboratory systems capability to conduct zoonotic disease detection in two facilities including the Ministry of Health and Sports' Department of Medical Research as well as the Ministry of Livestock, Agriculture, and Irrigation's Livestock Breeding and Veterinary Department. Scientists from both facilities were sent to the University of California, Davis as well as the Pasteur Institute in Cambodia, respectively, for advanced training in PREDICT diagnostic protocols. Through PREDICT, both facilities received deep freezers, thermocyclers, gel electrophoresis units, and all of the necessary items to conduct PREDICT project testing. Supply chains were created that benefitted local suppliers in Myanmar which continue to be used by the respective labs post-PREDICT.

PRACTICAL IMPLICATIONS & KEY BEHAVIORAL INTERVENTIONS

In Myanmar, the PREDICT project's activities have benefitted national and community zoonotic disease surveillance, preparedness and response. With the discovery of novel coronaviruses in Myanmar's bat populations, the One Health network was strengthened by incorporating wildlife and the environment into national planning for the prevention of emerging infectious diseases. PREDICT's lasting impact has created a road map for coordination and support between the three ministries for human and animal health and the environment, which together have a basis to conduct continued research and capacity building for further surveillance activities beyond the PREDICT project.

Through community engagement and participation across the project, PREDICT identified a desire of at risk-communities to learn how to protect themselves while appreciating the ecological benefit of coexisting with wildlife. For most, the PREDICT project's disease risk communication and strategies for living safely with animals was the first public health education they had received.

Although no evidence of exchange or sharing of viruses was identified between wildlife and humans, the discovery of novel viruses from priority high-risk virus groups in bats and rodents in caves frequently visited by people, including tourists, demonstrates a potential and previously unrecognized risk for disease transmission in Myanmar.

By investigating community behavior, the PREDICT team identified high-risk routine practices that correlate with human illness. Interventions emphasizing practical behaviors in industries with frequent and intimate interactions such as animal production, hunting and sanctuaries should be explored. Provision and use of protective gear or clothing (e.g. gloves, face masks) to prevent wounds and contact with infected animals would be effective recommendations to reduce occupational exposures.

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PREDICT
NEPAL
ONE HEALTH IN ACTION (2012-2020)

Enhancing One Health surveillance capabilities to detect (re)emerging diseases at the crossroads of Asia

PREDICT Nepal is the country's first novel zoonotic virus surveillance program using a One Health approach. This project has been implemented by the Center for Molecular Dynamics-Nepal (CMDN) in close partnership with national stakeholders, private partners, and local communities since 2012. This project has systematically collected human and animal samples from both rural and urban wildlife interfaces to detect, track, and better understand the emergence of new zoonotic pathogens from wildlife that could pose a threat to human health. The PREDICT Nepal team also collected information related to high-risk, animal-human interfaces as well as human activities and behavior with the potential to transmit and enhance spread of diseases. The PREDICT team established hospital-based syndromic surveillance, which enabled hospital assessments in addition to community-based surveillance.

PREDICT Nepal conducted seasonal, concurrent sampling of animals and humans in rural and urban communities as well as year-round, hospital-based syndromic sampling in clinical patients of Nepal. Altogether, 26,217 samples were collected from 3,293 animals (bats, birds, rodents and non-human primates) and 2,048 humans during eight years of the project. These samples were screened for five virus groups: paramyxoviruses, coronaviruses, influenza viruses, filoviruses, and flaviviruses. Behavioral data collected in the clinical and community settings will help identify potential behavioral risk factors that contribute to viral spillover.

Findings from the PREDICT project were shared with national stakeholders and local partners regularly through reports, workshops, and meetings with an aim to improve recommendations for disease surveillance and prevention. Through analysis of project data and findings, the PREDICT project was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

PREDICT is a revolutionary project that has strengthened the country's emerging disease surveillance capacity, bolstered the One Health platform, and promoted collaborative work between the stakeholders and scientific communities for evidence-based policy in Nepal. PREDICT also organized Nepal's first One Health stakeholders' workshop to identify and promote collaboration among government and private agencies. PREDICT contributed to further develop One Health capacity by organizing workshops such as "Zoonotic Disease Pandemic Preparedness for South Asia." In addition to developing technical and human capacity for emerging disease surveillance, PREDICT has supported the government in outbreak investigation and contributed to One Health policy briefs at national and regional levels.

LOCAL PARTNERS

- Teaching hospitals at Patan of Kathmandu Valley and Bharatpur of Chitwan District
- Communities of rural and urban study sites
- Department of Livestock Services, Ministry of Agriculture and Livestock Services
- Central Veterinary Laboratory, Ministry of Agriculture and Livestock Services
- Department of National Parks and Wildlife Conservation, Ministry of Forests and Environment
- Epidemiology and Disease Control Division, Ministry of Health

>60

DEVELOPED the One Health Workforce by training more than 60 people in Nepal.

>5.2K

OPERATIONALIZED One Health surveillance and sampled over 5.2K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Center for Molecular Dynamics Nepal/ Intrepid Nepal

52,151
TESTS

7
PREDICT

12
KNOWN

DETECTED 19 unique viruses in both animal and human populations.

RAJINDRA NAPIT

Senior Research Associate, *Center for Molecular Dynamics Nepal*

"PREDICT's One Health Approach has been a great experience for me. I have been trained in biosafety, field epidemiology and surveillance and laboratory diagnostics and screening of various emerging pathogens using PCRs with high throughput sequencing. Using developed and optimized PREDICT protocols, we have been able to detect some emerging pathogens. PREDICT gave [me an] opportunity to work in community setting[s] with exposure from sample collection to final reporting. It has helped me understand the dynamics of zoonotic viral spillover, evolution, prevention and measures and practices that reduce disease transmission risk."

DHIRAJ PURI

Field Officer, *Center for Molecular Dynamics Nepal*

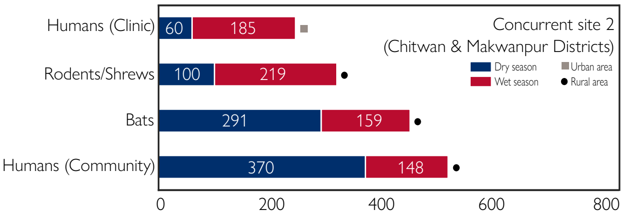
"As a vet technician, initially I [had] knowledge on poultry. But with PREDICT, I expand[ed] my knowledge about primates, rodents, ducks, bats, use of PPE, and skills on handling and collecting samples from these animals. I am also able to use [the EIDITH software] to enter and analyze sample data. In a word, PREDICT plays [a] vital role to increase my working area and [I] would like to thank [them] for providing [these] opportunities."

ACHIEVEMENTS

- PREDICT Nepal has generated the largest collection of animal (bats, birds, rodents, and non-human primates) and human specimens in Nepal virus surveillance history.
- PREDICT enhanced characterization of fevers of unknown origin in patients by collaborating with healthcare workers at hospitals in two concurrent study sites.
- PREDICT supported Nepal's first workshop, "Feasibility of One Health (OH) initiative in Nepal for surveillance of emerging and re-emerging zoonotic diseases of public health importance."
- PREDICT Nepal expanded antimicrobial resistance (AMR) testing and established proof-of-concept for a One Health approach to AMR surveillance by which animal, human, and environmental samples are concurrently obtained to investigate AMR patterns in a community with intensifying livestock production.
- PREDICT Nepal established working relationships with the government agencies and local partners to implement a One Health project and integrate reporting across human, animal, and environmental health platforms.
- PREDICT assisted in drafting the National Strategies for Wildlife Disease Control through its partnerships with local government and private stakeholders by providing assistance in organizing and implementing a cross-disciplinary workshop focused on wildlife policy, "Strategic Planning Workshop for Improving Wildlife Health Capacity in Nepal" in 2016.
- PREDICT Nepal further supported One Health platforms and pandemic preparedness in the country by facilitating One Heath research in South Asia by implementing another workshop, "Zoonotic Disease Pandemic Preparedness for South Asia," in 2017.
- The project also assisted the government in strain characterization during the H5N8 influenza outbreak in 2017.

ONE HEALTH SURVEILLANCE

CHITWAN & MAKWANPUR

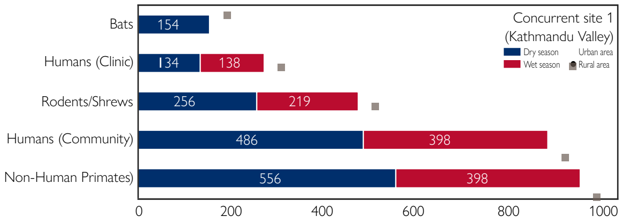


Numbers of individual taxa sampled during dry and wet seasons in Chitwan and Makwanpur Districts

Our team also conducted surveillance in a rural area south of the Kathmandu Valley in Makwanpur District inhabited primarily by the Chepang ethnic community. The Chepang traditionally farm and raise livestock and animals, but also hunt bats and are engaged in the wildlife value chain, selling animals or animal products to wildlife restaurants and markets. For syndromic surveillance, we worked with a teaching institution at Chitwan District that serves areas including Makwanpur.

The PREDICT project's One Health surveillance approach was designed to train, equip, and enable the workforce to collect data and build the evidence base for zoonotic diseases and emerging viral threats in vulnerable areas. PREDICT is the first systematic project in Nepal to use the One Health approach for virus surveillance in both animal and human populations. Our team safely and humanely sampled wildlife, domestic animals, and people concurrently, targeting high-risk communities for viral spillover and spread in urban and rural areas of Nepal. In addition, we implemented syndromic surveillance of febrile patients at major hospitals near our at-risk surveillance sites. We also used molecular tools to confirm the species of sampled wildlife (30 bats and 146 rodents), and identified five species of rodents along with three species of bats.

KATHMANDU VALLEY



Numbers of individual taxa sampled during dry and wet seasons in Kathmandu Valley

Our team conducted surveillance at low-income informal settlements situated along urban rivers in Kathmandu. At these sites, residents raise animals, grow food in urban gardens, and engage in a variety of livelihood activities. These sites are considered vulnerable due to the close contact between animals and people and lack of infrastructure, improved sanitation, and availability of health services. In addition, we sampled macaques at two temple heritage sites that attract local and international tourists. For syndromic surveillance, our team worked with a public not-for-profit tertiary academic institution that receives patients from all over the country.

VIRUS DETECTION

Virus discovery efforts have an important role to play in preparing for future epidemics. These activities are contributing to a global health paradigm shift by incorporating a more proactive approach to surveillance and pandemic preparedness. By finding viruses in wildlife before they emerge in humans, we stand to learn more about the ecology and evolution of virus diversity and transmission risk of potentially pandemic strains.

The PREDICT project's virus detection uses broadly reactive consensus (genus/family level) PCR supplemented with high throughput sequencing. These powerful tools produce specific, high-resolution data, allowing for detection of known and new potential pathogens. Between 2012 and 2019, 19 unique viruses were detected in animals and people.

In Nepal, our findings of novel paramyxoviruses and coronaviruses in rodents are good examples of how we are operationalizing this proactive paradigm. During the first phase of the project (2012-2014), we sampled wildlife in urban areas of Kathmandu and detected coronaviruses, paramyxoviruses, hantaviruses, and rhabdoviruses in rodents and shrews; henipaviruses and retroviruses in macaques; and coronaviruses in bats.

In the second phase of the project (2014-2019), we sampled animals and people in Kathmandu Valley, as well as in Chitwan and Makwanpur Districts. We detected a total of 10 unique viruses belonging to 4 virus groups (coronaviruses, paramyxovirues, flaviviruses, and influenza viruses) within humans, rodents, shrews, and mallards.

VIRUS TABLE

VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	Alphacoronavirus NL63	Human	Makwanpur District	2	2	0
	Coronavirus 229E (Human strain)	Human	Kathmandu Valley	2	0	2
	Murine coronavirus	Black Rat	Kathmandu Valley	3	0	3
	Duck coronavirus	Mallard	Kathmandu Valley	16	0	16
	Infectious bronchitis virus	Mallard	Kathmandu Valley	3	0	3
Paramyxovirus	Human parainfluenzavirus 3	Human	Makwanpur District	1	0	1
	PREDICT_PMV-83	Asian House Shrew	Kathmandu Valley	3	0	3
	Newcastle disease virus	Mallard	Kathmandu Valley	8	0	8
Influenza virus	Influenza A	Human, Mallard	Kathmandu Valley, Clinic (Kathmandu), Makwanpur District	21	4	17
Flavivirus	Dengue virus serotype 2	Human	Clinic (Chitwan)	1	0	1
Total				**60**	**6**	**54**

EPIDEMIOLOGIC & BEHAVIORAL RISK

The PREDICT project deployed a multidisciplinary One Health surveillance approach, working with local communities to evaluate animal-human interactions, animal distributions, human demographics, behaviors, and activities at sampling sites. Between September 2016 and September 2018, 2,046 individuals participated in clinic and community-based surveillance activities. Samples were collected from all participants to screen for viruses, and interviews were conducted to collect survey data to shed light on potential drivers and risk pathways for zoonotic viral spillover and spread. Both epidemiological and ethnographic methods were used in data collection and analysis to generate insights.

PARTICIPANT CHARACTERISTICS ACROSS ALL SURVEILLANCE SITES

	Clinic-based surveillance (n=543)		Community-based surveillance (n=1,503)	
Study Sites	Clinic (Kathmandu) (n=275)	Clinic (Chitwan) (n=268)	Urban (Kathmandu) (n=946)	Rural (Makwanpur) (n=557)*
Gender				
Female	117 (42.5%)	134 (50.0%)	604 (63.8%)	336 (60.3%)
Male	158 (57.5%)	134 (50.0%)	342 (36.2%)	221 (39.7%)
Age (years)				
0-10	38 (14.2%)	56 (20.4%)	80 (8.5%)	72 (12.9%)
11-20	49 (18.3%)	57 (20.7%)	367 (38.8%)	203 (36.4%)
21-40	86 (32.1%)	75 (27.3%)	240 (25.4%)	104 (18.7%)
41-60	63 (23.5%)	53 (19.3%)	77 (8.1%)	55 (9.9%)
61+	32 (11.9%)	34 (12.4%)	182 (19.2%)	123 (22.1%)

*2 did not participate in the behavior survey

CLINIC-BASED SURVEILLANCE

Clinic-based surveillance aimed to detect viruses causing illness in people. Specifically, we targeted patients with undifferentiated or undiagnosed fever (temperature ≥ 38°C (100.4°F)) and tested individuals for coronaviruses, filoviruses, flaviviruses, influenza viruses, and paramyxoviruses.

At the time of sampling, most participants had acute fever of 1-5 days' duration. In addition to fever, patients reported a range of other symptoms, including cough (30.9%), diarrhea and/or vomiting (41.6%), headache and/or joint pain (77.7%), convulsions and/or altered consciousness (2.9%) or a combination of these symptoms. Fever with cough was more common in patients at the Kathmandu clinic (73.8%) compared to patients sampled at the Chitwan clinic (26.2%). Symptoms did not vary by gender and age-class of patients sampled, except for fever with headache and/or joint pain, which was significantly more common in patients >11 years old.

Participants enrolled in the hospital setting were most commonly students (Kathmandu Clinic: 36.4%; Chitwan Clinic: 32.8%), agricultural workers (Kathmandu Clinic: 25.0%; Chitwan Clinic: 22.9%), non-animal business workers (Kathmandu Clinic: 19.6%; Chitwan Clinic: 12.7%), or homemakers (Kathmandu Clinic: 12.7%; Chitwan Clinic: 27.6%). Among agricultural workers sampled, the majority were involved in crop production (48.4%) or did both animal and crop production (40.0%), and the remaining were engaged in animal production alone.

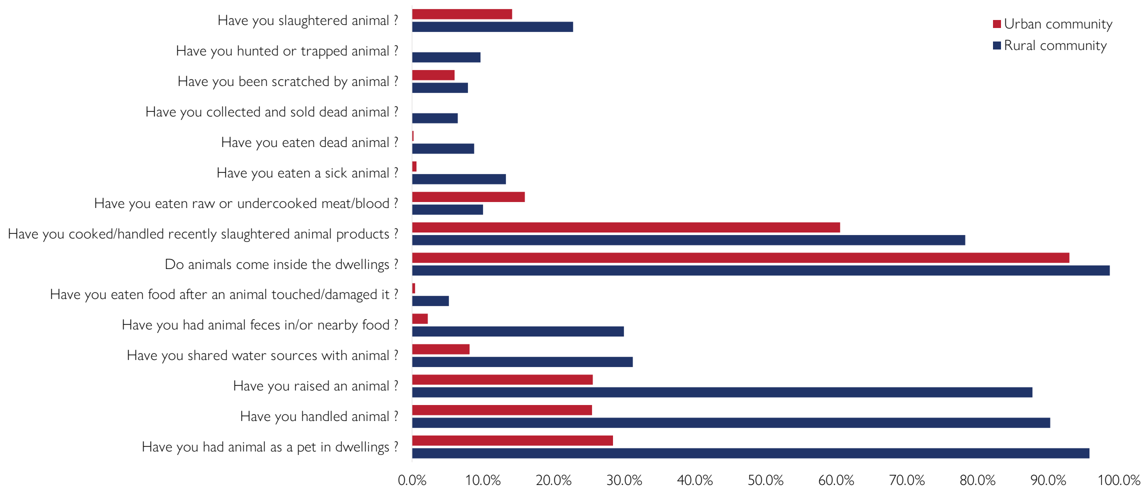
COMMUNITY-BASED SURVEILLANCE

From 2016 to 2018, 1,503 individuals participated in One Health surveillance and behavioral risk investigations at community-based surveillance sites in urban Kathmandu and in rural areas. The majority of participants from the rural community were involved in both animal and crop productions whereas urban participants were mostly involved in animal production. There were significantly higher numbers of unemployed people sampled in the urban community.

However, more individuals reported not attending school in the rural community compared to the settlement in Kathmandu; therefore literacy rates in this area may be lower. Among people interviewed, contact with both wild and domestic animals was prevalent in both rural and urban communities. Participants involved in crop and animal production reported crop foods raided by rodents and shrews (18%), non-human primates (54%), and other wild animals.

The majority of people in rural and some from urban communities reported that they had eaten raw, sick animals, or animals found dead. Hunting of wild animals and selling of dead animals were observed only in the rural study site, where communities engaged in hunting were targeted for surveillance and where residents reported hunting, cooking, or handling bats. Other animals were also hunted in this community to mitigate crop and food raiding. Hunting of bats was most common in male and adult (20-60 years) participants. People who hunted bats were also more likely to report influenza-like symptoms in the past year, compared to individuals who did not hunt bats. Respondents from the rural community provided important context for their contact with animals, including bats, in ethnographic interviews and focus group discussions.

In both communities, our findings highlight concerns over sanitation and hygiene, lack of knowledge on disease risks, and the prevalence of high-risk activities for viral spillover related to animal-human interactions. These behaviors and documented knowledge gaps show vulnerabilities to infectious diseases. At both sites, meetings were held in partnership with local authorities to raise awareness of zoonotic diseases and to identify potential strategies for risk reduction and disease prevention.



Responses on animal contact compared across rural and urban community participants

SELECTED EXCERPTS ON ANIMAL CONTACT AND HUNTING FROM ETHNOGRAPHIC INTERVIEWS AND FOCUS GROUP DISCUSSIONS:

“Who usually takes care of animals in the household?”

> Most of the time, we women take care of animals at home as men are mostly in the field to farm, but they sometimes help to feed animals. Men slaughter the animals.

“Do you hunt/capture wild animals that you have said are seen in this area? Do you also hunt or consume them?”

> Ancestors used to hunt bats and we also learned from them. If the elder of the family hunts, then their family eats bats. Some occasionally sell them at the market. Bat tastes like pigeon meat. We do not eat the small bats that eat insects.

> I have not hunted or eaten bats as there are no ‘Chiuri’ plants in our area but we hunt ‘Gosya’ a cat-like animal that eats fruit.

RISK COMMUNICATION

Given the zoonotic risk identified in bat species from surveillance activities conducted throughout the PREDICT project, communities in the rural study sites were informed about the risks associated with bat hunting and consumption.

Outreach and risk reduction campaigns were also conducted after the completion of field work through leaflets and booklets including PREDICT's behavior change communication and risk reduction resource, *Living Safely with Bats*. These efforts helped increase awareness of the potential dangers of direct contact with bats and helped identify strategies for staying safe from exposure and infection, while ensuring the health and safety of the bats.

COMMUNITY ENGAGEMENT

By design, the PREDICT team engaged local communities to forge partnerships, build trust, and develop participatory One Health surveillance. Through these collaborations, local individuals are recruited to act as ambassadors and liaisons, and speak with their communities about zoonotic diseases, transmission dynamics, risky behaviors and practices, and measures to mitigate risk. In these communities, PREDICT also improved access to healthcare, linking vulnerable populations with health professionals and needed services. In addition, the PREDICT team shared resources and strategies with community partners of laboratory findings and surveillance summaries designed to reduce risks for virus tranmission while balancing health and conservation goals.

STRENGTHENING CAPACITY

Through hands-on trainings and experiential learning embracing the One Health approach to disease surveillance and detection, the PREDICT project has strengthened capabilities in Nepal to conduct behavior risk investigations, humanely and safely collect samples from animals and humans, safely transport samples to laboratories for testing, detect viruses and emerging disease threats, and develop disease prevention and risk communication strategies.

Since 2012, PREDICT has trained >65 individuals from multiple disciplines, including doctors, veterinarians, field biologists, epidemiologists, molecular biologists, sociologists, community leaders, para-veterinarians, and government officials. In addition, our team has trained 50 members of the global health workforce (20 interns and 30 visiting international students). Trainings were conducted through conferences, hands-on field and lab experiences, internships, and workshops.

PRACTICAL IMPLICATIONS

- The PREDICT project's work in Nepal has been integral to establishing emerging viral disease surveillance using the One Health approach and strengthening One Health platforms in the country.
- Pilot studies conducted by PREDICT in partnership with hospitals in Nepal have highlighted the importance of screening for emerging and re-emerging viruses in clinical settings.
- Surveillance activities involving bats, rodents, and primates have strengthened the wildlife health capacity in Nepal and expanded the workforce with knowledge and skills needed to work with wildlife.

For more information view the interactive report at **p2.predict.global**

PREDICT THAILAND

ONE HEALTH IN ACTION (2009-2020)

10 years of PREDICT activities in Thailand has developed leaders in the One Health approach to pandemic preparedness, outbreak response, and virus discovery.

THAILAND

Thailand is a biologically diverse country where ecological changes caused by land conversion and agricultural production are bringing humans into increased contact with wildlife and the pathogens they carry. A diversity of novel wildlife viruses with potential economic and public health importance are circulating in Thailand while the threat posed by pathogens like Nipah virus, coronaviruses, and influenza virus highlights the urgent need to support ongoing research, surveillance, and capacity building efforts. Local community interactions with wildlife in rural settings (e.g. with macaques or flying foxes in temples, or with bats while harvesting guano for fertilizer) represent additional interfaces for zoonotic disease spillover. Bangkok and other areas of Thailand are hubs for the wildlife trade and regional centers of travel and tourism. Thailand is centrally connected via porous borders with Lao PDR, Cambodia, Malaysia, and Myanmar. The high volume of cross-border movement of people, their animals, and trafficked wildlife poses an added risk for transboundary pathogen introduction and spread, including infected travelers bringing in MERS-CoV, Zika virus, Ebola virus, and SARS-CoV-2.

PREDICT in Thailand is led by the team at the World Health Organization (WHO) Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University and the Thai Red Cross Emerging Infectious Diseases Health Science Centre, in collaboration with the Faculty of Forestry at Kasetsart University, the Department of National Parks, Wildlife and Plant Conservation, and additional government partners in human and animal health. The project leverages these groups' expertise discovering novel viruses in humans and animals, and has increased these capabilities, especially for surveillance of specific pathogens. Capacity building is essential to the sustainability of the program. PREDICT project activities helped build the skills, systems, infrastructure, and human resources for emerging infectious disease preparedness within the teams and institutions involved in the project, and on a larger scale as these skills and practices have been adopted by additional government partners.

Ten years of PREDICT project activities helped strengthen an early warning 'One Health' system for emerging infectious disease (EID) preparedness capacity in Thailand, as implementing partners gained expertise in safe and humane wildlife sampling, human sample collection for unknown disease detection, behavioral risk research, laboratory testing and cold chain, and disease modelling and data analysis.

In addition, through analysis of project data and findings, the PREDICT team was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats. PREDICT project PCR protocols have been widely implemented to identify known pathogens of international concern (e.g. MERS, Ebola, SARS-CoV-2) upon their first introduction to Thailand, and to characterize and genotype strains of known and novel viruses from people and animals. Due to its abundant experience diagnosing EID viruses over the past 10 years, PREDICT Thailand's laboratory has been designated the Ministry of Public Health's reference laboratory for identification of EIDs. The first imported MERS case in Thailand in 2015 was identified and confirmed by the PREDICT lab team in country within 24 hours after receiving the specimen. During the Ebola outbreak in Africa in 2014, a PREDICT project protocol was used to test human biological samples from suspected imported cases in Thailand during the first few months when the standard diagnostic assay was not available in country. The PREDICT project in Thailand has also facilitated collaborations both nationally (Thai National Science and Technology Development Agency (NSTDA) and Thailand Research Fund) and internationally (United States Defense Threat Reduction Agency's Biological Threat Reduction Program (US-DTRA BTRP) to achieve EID monitoring and preparedness in the country. Thailand is a success story for capacity strengthening within the PREDICT project and has become a regional leader in laboratory diagnostics and training – working with partners in Myanmar, Lao PDR, Malaysia, Indonesia and beyond regularly exchanging information and leading joint lab and field training activities.

LOCAL PARTNERS

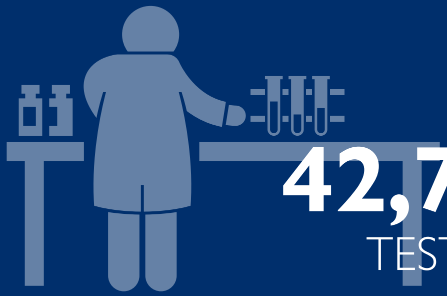
- WHO Collaborating Centre for Research and Training on Viral Zoonoses
- Thai Red Cross Emerging Infectious Diseases Health Science Centre
- Faculty of Medicine, Chulalongkorn University
- Faculty of Forestry and Faculty of Veterinary Medicine, Kasetsart University
- Thai Department of National Parks, Wildlife and Plant Conservation
- Thai Department of Disease Control, Ministry of Public Health
- Thai Department of Livestock and Development
- Office of Disease Prevention and Control 5
- Loei Hospital
- Health Promoting Hospital, Tambon Wat Luang
- Office of Khao Chong Phran Non-hunting Area
- Phu Luang Wildlife Research Station

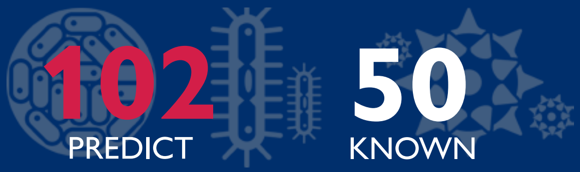
DEVELOPED the One Health Workforce by training more than 310 people in Thailand.

OPERATIONALIZED One Health surveillance and sampled over 4.7K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- WHO-CC Viral Zoonoses Chulalongkorn University





DETECTED 152 unique viruses in both animal and human populations.

SUPAPORN WACHARAPLUESADEE
Country Coordinator & Laboratory Chief, *WHO-CC Viral Zoonoses Chulalongkorn*

"PREDICT is a live exercise program. PREDICT is a One Health University without degree but is so much more. PREDICT is country-strengthening. PREDICT is not just a program, it promotes country's success on EID preparedness. PREDICT is more than just predicting. Without PREDICT, Thailand may have been slow on handling the response to Ebola, MERS and COVID-19 introductions. PREDICT has helped us to gain experience, make unknown pathogens known, and build partnerships and trust."

THANAPOL PHUTTHARAKSA
Head of Village Health Volunteers, *Chonburi Province*

"PREDICT has been very beneficial for communities. Before TRC-EID implemented the PREDICT project in our community, we were not even aware that bats harbored infectious pathogens."

ACHIEVEMENTS

- Early detection of imported human cases of MERS and SARS-CoV-2, and screening for suspected Ebola cases, using PREDICT project diagnostic protocols.
- Trained over 300 people in core skills required for zoonotic disease surveillance and detection, including rangers and staff from the government Department of National Parks, Wildlife, and Plant Conservation.
- Collected more than 36,000 specimens from >4,700 individual animals and people at high-risk interfaces throughout 10 years of PREDICT project activities.
- Discovered 102 new viruses and detected 40 previously-identified viruses in virus groups with pandemic potential from bats, rodents, and primates throughout 10 years of PREDICT project activities.
- Conducted behavioral risk research to identify zoonotic disease risk factors and trained field staff from the Ministry of Public Health on best practices for in-depth behavioral risk investigations.
- Trained laboratory personnel from the Ministry of Public Health, Department of Disease Control on using PREDICT protocols for novel virus detection.
- Collaborated with the Department of National Parks, Wildlife, and Plant Conservation (DNP) in the investigation of a wildlife die-off event in Kanchanaburi Province.
- Led a health screening for the 'Cave Boys' rescued soccer team to test for exposure to multiple pathogens using PREDICT and other protocols to identify potential zoonoses.
- Expanded local community health engagement to include zoonotic disease awareness and prevention at multiple sites.

ONE HEALTH SURVEILLANCE

The PREDICT team conducted concurrent biological surveillance activities at high-risk animal-human interfaces in the country, focusing on in-depth 'One Health' investigations at three sites over the last 5 years. Putting One Health into action, the team successfully coordinated with local health facilities and the government Thai Department of Livestock Development (DLD) to conduct triangulated human, wildlife, and domestic animal surveillance. While the PREDICT team safely collected samples from wildlife (bats, rodents, and non-human primates) and humans in collaboration with local Ministry of Public Health and Community Health Volunteer staff. PREDICT also conducted behavioral risk investigations among healthy humans living in these same sampled communities. In partnership with the Food and Agriculture Organization (FAO), DLD staff collected samples from pigs and other domestic animals alongside PREDICT. DLD staff were then trained on PREDICT project diagnostic PCR protocols and data entry processes using the EIDITH program. This collaboration is a great example of how the project helped bring teams from different sectors together to efficiently and effectively conduct One Health disease surveillance.

The PREDICT team selected sites for One Health surveillance that included interfaces associated with land use change, wildlife consumption, guano harvesting, and human dwellings sited near large wildlife populations in three provinces of Thailand:

- **Loei Province**: Loei Province is a typical provincial rural area from northeastern Thailand in the midst of land use conversion, as forest is cleared for production of rice, corn, and cassava. It is also the site of an active rodent trade, as rodent consumption is common. Bats and rodents in and around land conversion areas, human dwellings, and markets were sampled. The PREDICT team also worked with the provincial hospital in Loei to conduct surveillance of patients with hemorrhagic and encephalitic symptoms.
- **Ratchaburi Province**: Ratchaburi Province is in the western part of Central Thailand. Karst (limestone mountain) formations and caves are common, and our surveillance sites included caves where bat guano is collected by hand and sold for local agricultural production. PREDICT previously identified Betacoronavirus Group C (MERS-CoV group) viruses from bat guano collected from this region. Bat guano miners and people working in the bat guano fertilizer trade (sellers, buyers, middle men, handling workers), all high-risk occupations for disease transmission, were targeted for behavioral risk interviews and biological sample collection. Bats, rodents, and non-human primates are abundant at these sites that include tourist attractions and were also sampled as part of our effort to investigate virus sharing between the closely linked human and wildlife populations. Livestock and domestic animals were also sampled around bat roost temples and villages in collaboration with FAO and DLD.
- **Chonburi Province**: Chonburi is a province in Central/Eastern Thailand where there is extensive swine production and mixed agricultural production. Large fruit bat colonies exist in this province where Nipah virus has been previously identified. Fruit bat foraging areas overlap with pig production sites, and the Buddhist temple that harbors bat populations is a local site of worship and a tourist attraction. Fruit bats in the communities located near pig farms were sampled by the PREDICT team while pigs were sampled in collaboration with FAO and DLD partners. A local health promotion clinic, directly opposite a large fruit bat roost, was the site for human community surveillance of people living or working close to bat roosts.

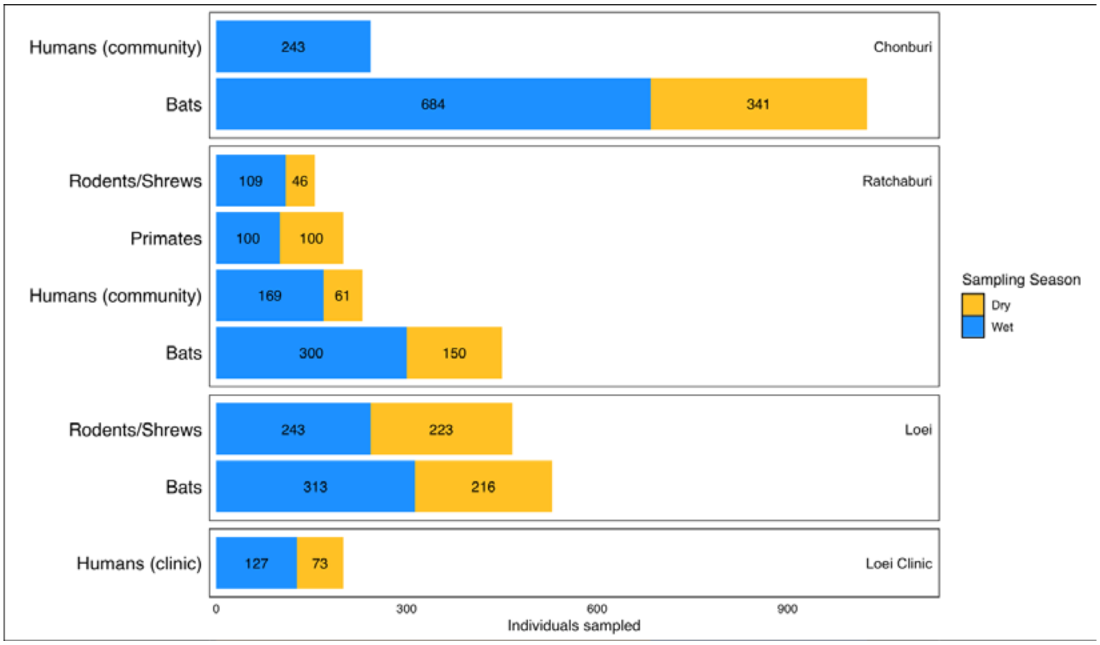


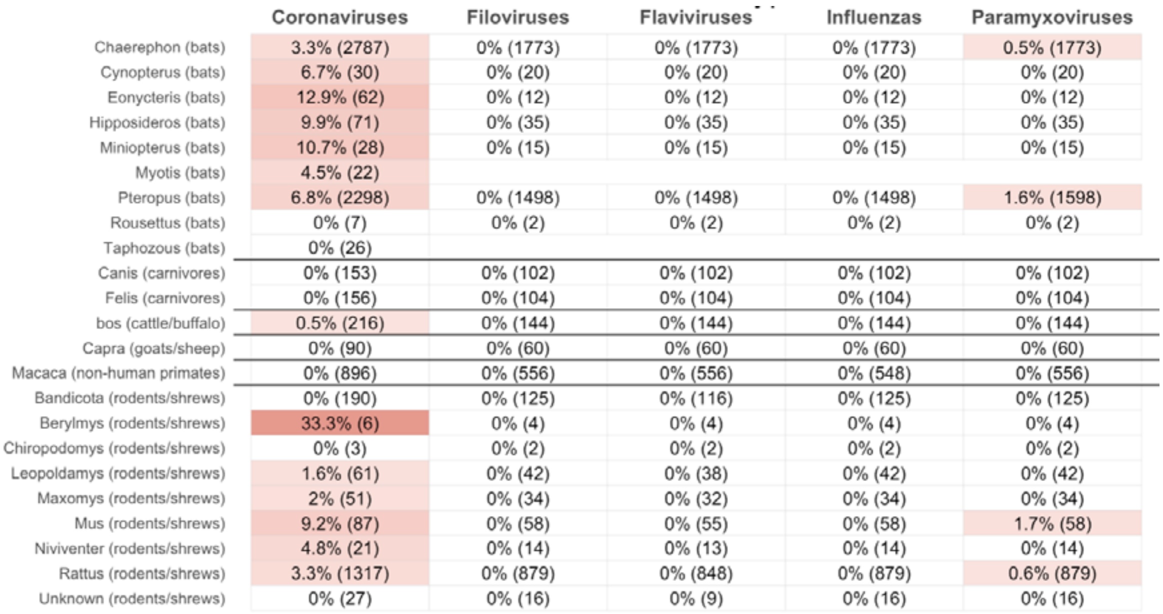
FIGURE 1. ***Summary of the PREDICT project's One Health sampling efforts at each surveillance site in Thailand.***

VIRUS DETECTION

VIRUS FINDINGS IN ANIMALS

The PREDICT project's strategy for virus detection included screening samples using broadly reactive consensus PCR (cPCR) for priority virus groups, including coronaviruses, filoviruses, flaviviruses, and paramyxoviruses as well as influenza virus. Positives detected using these assays were sequenced to identify the viruses and compare their relationship to known pathogens, and viruses were prioritized for further characterization. This approach allows for detection of both known and novel viruses and improves our understanding of the presence and diversity of viruses, as well as potential pathogens, in humans and animals.

Using this approach, the PREDICT team safely tested 5,397 bat, 1,696 rodent, and 896 non-human primate oral and rectal swab samples. Of these 7,989 specimens tested, 263 were positive for coronaviruses (217 bats and 46 rodents), and 36 for paramyxoviruses (30 bats and 6 rodents). Significant coronavirus findings in wildlife included the discovery of SARS-related coronaviruses in roundleaf bats (*Hipposideros* spp.); MERS-related viruses discovered at a bat guano harvesting human interface; identification of porcine epidemic diarrhea virus in bats; four novel bat coronaviruses detected more than 30 times each; and a diversity of other new coronaviruses from both bats and rodents. Significant paramyxovirus findings in wildlife included multiple Nipah virus detections; several new rodent species recognized as hosts for murine coronavirus – expanding the known host range; and detection and characterization of 15 new paramyxoviruses discovered in bats and rodents. No filoviruses, flaviviruses, or influenza viruses were detected from any wildlife specimens. For details on detections and testing effort for each virus group and host genus combination, see heatmaps and tables on the following page.



	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
Chaerephon (bats)	3.3% (2787)	0% (1773)	0% (1773)	0% (1773)	0.5% (1773)
Cynopterus (bats)	6.7% (30)	0% (20)	0% (20)	0% (20)	0% (20)
Eonycteris (bats)	12.9% (62)	0% (12)	0% (12)	0% (12)	0% (12)
Hipposideros (bats)	9.9% (71)	0% (35)	0% (35)	0% (35)	0% (35)
Miniopterus (bats)	10.7% (28)	0% (15)	0% (15)	0% (15)	0% (15)
Myotis (bats)	4.5% (22)				
Pteropus (bats)	6.8% (2298)	0% (1498)	0% (1498)	0% (1498)	1.6% (1598)
Rousettus (bats)	0% (7)	0% (2)	0% (2)	0% (2)	0% (2)
Taphozous (bats)	0% (26)				
Canis (carnivores)	0% (153)	0% (102)	0% (102)	0% (102)	0% (102)
Felis (carnivores)	0% (156)	0% (104)	0% (104)	0% (104)	0% (104)
bos (cattle/buffalo)	0.5% (216)	0% (144)	0% (144)	0% (144)	0% (144)
Capra (goats/sheep)	0% (90)	0% (60)	0% (60)	0% (60)	0% (60)
Macaca (non-human primates)	0% (896)	0% (556)	0% (556)	0% (548)	0% (556)
Bandicota (rodents/shrews)	0% (190)	0% (125)	0% (116)	0% (125)	0% (125)
Berylmys (rodents/shrews)	33.3% (6)	0% (4)	0% (4)	0% (4)	0% (4)
Chiropodomys (rodents/shrews)	0% (3)	0% (2)	0% (2)	0% (2)	0% (2)
Leopoldamys (rodents/shrews)	1.6% (61)	0% (42)	0% (38)	0% (42)	0% (42)
Maxomys (rodents/shrews)	2% (51)	0% (34)	0% (32)	0% (34)	0% (34)
Mus (rodents/shrews)	9.2% (87)	0% (58)	0% (55)	0% (58)	1.7% (58)
Niviventer (rodents/shrews)	4.8% (21)	0% (14)	0% (13)	0% (14)	0% (14)
Rattus (rodents/shrews)	3.3% (1317)	0% (879)	0% (848)	0% (879)	0.6% (879)
Unknown (rodents/shrews)	0% (27)	0% (16)	0% (9)	0% (16)	0% (16)

FIGURE 2. *Heatmaps providing a summary of sampling effort and detection of positives for the five priority virus groups: coronaviruses, filoviruses, flaviviruses, influenzas, and paramyxoviruses. The data are summarized by host at the genus level, and broader taxonomic groups are shown in parentheses. The heatmap data show the percentage of virus positives that were confirmed by sequencing (%) and the number of PCR tests performed (in parentheses). The red color scales with increased virus positives. Host taxa or interfaces that did not test positive are shown in white.*

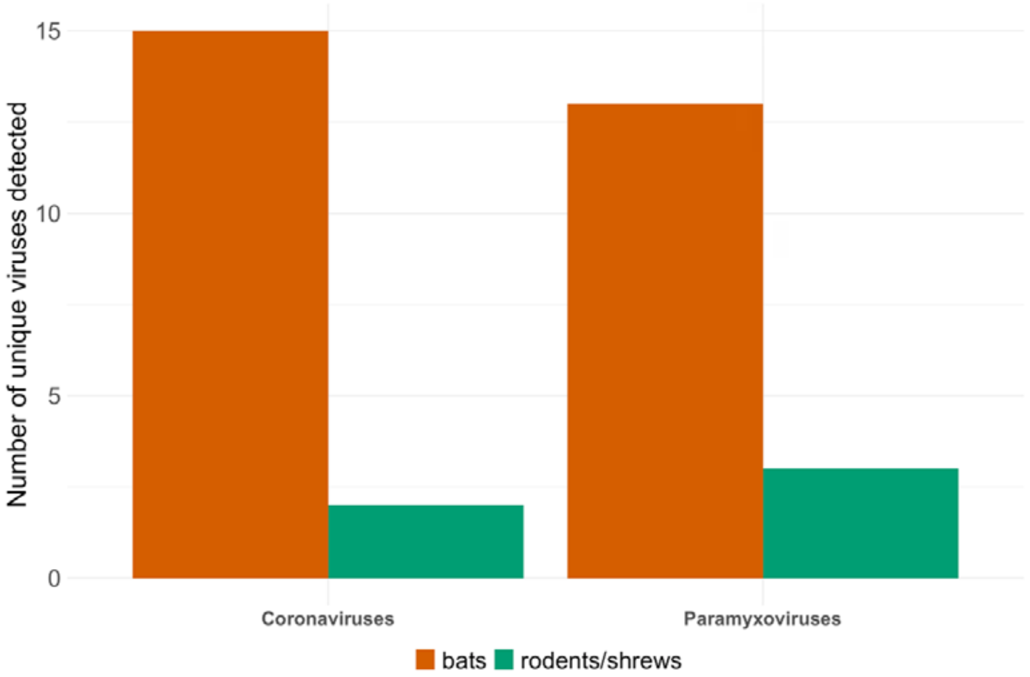


FIGURE 3. *Number of unique coronaviruses and paramyxoviruses detected in bats and rodents in Thailand.*

In addition to characterizing a number of newly discovered viruses from wildlife, another outcome of PREDICT sampling and testing was expanding the known host range for previously described viruses. For example, 20 new rodent host species were discovered for murine coronavirus from the PREDICT project sampling efforts, six of these from Thailand. Host range information is important for helping predict spillover potential of viruses, as viruses with a large "breadth" of different hosts are more likely to infect people – as was shown in previous analyses from the PREDICT project (Olival et al., 2017; Johnson et al., 2015).

VIRUS FINDINGS IN PEOPLE

Through partnerships with national and community-level public health experts the PREDICT team conducted both community and clinic-based surveillance for emerging viruses in people. Nine known human pathogens were detected during human surveillance activities, with a majority discovered during hospital and local clinic-based surveillance (Table 1). The most common virus detected was influenza virus, including both influenza A and B strains, but notable paramyxoviruses including human parainfluenza virus 1 and Measles virus were also identified from clinical specimens. Influenza A and B strains were detected in approximately 50 patients from Loei hospital, and interestingly the virus was detected in nasopharyngeal swabs and in many cases also in rectal specimens from the same individuals, or in rectal specimens only (Figure 4, Sangkakam, In Review). This finding has relevance for designing passive surveillance of human influenza strains, i.e. through non-invasive testing of sewage systems.

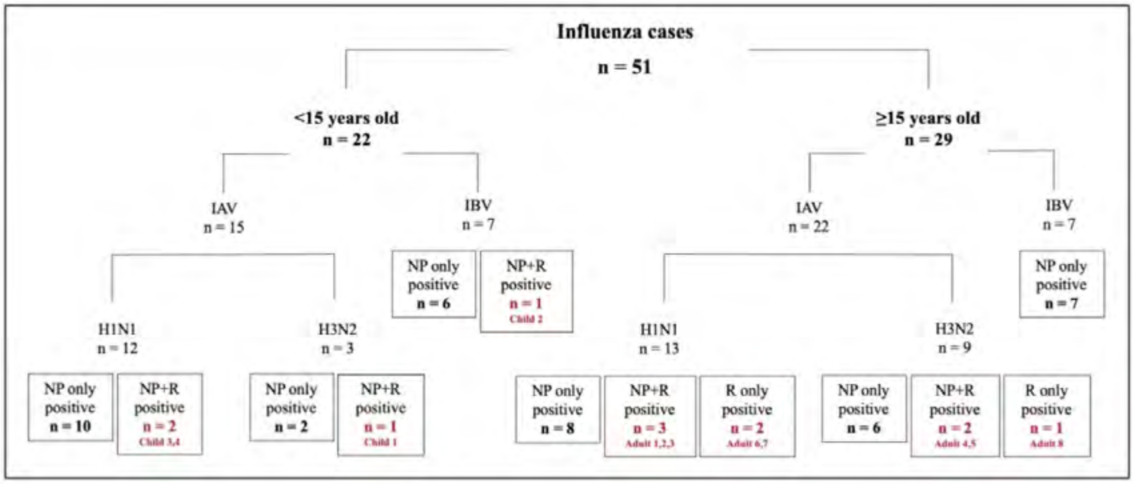


FIGURE 4. ***Summary of approximately 50 influenza positive specimens (IAV = Influenza A virus; IBV = Influenza B virus) from clinical surveillance at Loei hospital, showing influenza detection in both nasal (NP) and rectal (R) specimens (Sangkakam et al., In Review).***

The PREDICT team provided critical support to the Ministry of Public Health (MOPH) and helped facilitate the early detection of imported emerging human viruses to Thailand. This included the rapid detection of MERS-CoV, suspected cases of Ebola virus during the West Africa outbreak, and COVID-19 in one of the earliest cases of spread out of Wuhan, China using PREDICT PCR protocols. The PREDICT and MOPH teams reported detecting the first MERS-CoV case in Thailand which was later published in the Eurosurveillance journal, August 2017. This success story highlights how using multiple coronavirus and MERS-specific assays at once allowed for rapid detection and coronavirus sequence confirmation within 24 hours, thus halting local transmission.

Other success stories from human surveillance in Thailand include supporting the MOPH´s national surveillance program for hand, foot, and mouth disease. In this case, the PREDICT lab used the project's Enterovirus PCR protocols for testing patient specimens on behalf of the MOPH. Also, as part of national guidelines from the Bureau of Epidemiology, Department of Disease Control, MOPH, PREDICT project protocols (human sampling and PCR) were used in collecting and testing samples from the youth soccer team trapped in the Tham Luang cave and their rescuers. Collected samples were tested at PREDICT laboratories.

A notable finding from community-based surveillance was the detection of human coronavirus HKU1 (HCoV-HKU1) in a bat guano miner (harvester). The PREDICT Thailand team was able to sequence, characterize, and publish this finding in 2019 (Joyjinda et al., 2019). This virus was detected from an individual with a high level of occupational exposure to bat feces, but who was asymptomatic at the time of sampling. While this individual was likely exposed to HCoV-HKU1, a fairly common human coronavirus, due to person-to-person transmission and not via exposure from bats, our surveillance strategy and virus characterization pipeline provide valuable insight into the circulation of endemic infectious diseases in Thailand and increase the country's preparedness for other novel emerging infectious diseases.

TABLE 2. VIRUS DETECTIONS IN HUMANS BY SITE AND SPECIMEN TYPE.

VIRUS	VIRUS GROUP/ GENUS	# OF DETECTIONS	SITE	SPECIMEN TYPES
BETACORONAVIRUS 1 (OC43)	CORONAVIRUSES	2	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB
HUMAN CORONAVIRUS HKU1	CORONAVIRUSES	1	RATCHABURI	NASOPHARYNGEAL SWAB
DENGUE VIRUS	FLAVIVIRUSES	1	LOEI HOSPITAL	BLOOD (WHOLE)
ZIKA VIRUS	FLAVIVIRUSES	1	CHULALONGKORN HOSPITAL	URINE
INFLUENZA A	INFLUENZA VIRUSES	46	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB
INFLUENZA B	INFLUENZA VIRUSES	10	LOEI HOSPITAL	NASOPHARYNGEAL SWAB
HUMAN ENTEROVIRUS B	ENTEROVIRUSES	2	LOEI HOSPITAL	RECTAL SWAB
HUMAN PARAINFLUENZA VIRUS 1	PARAMYXOVIRUSES	9	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB
MEASLES VIRUS	PARAMYXOVIRUSES	2	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB

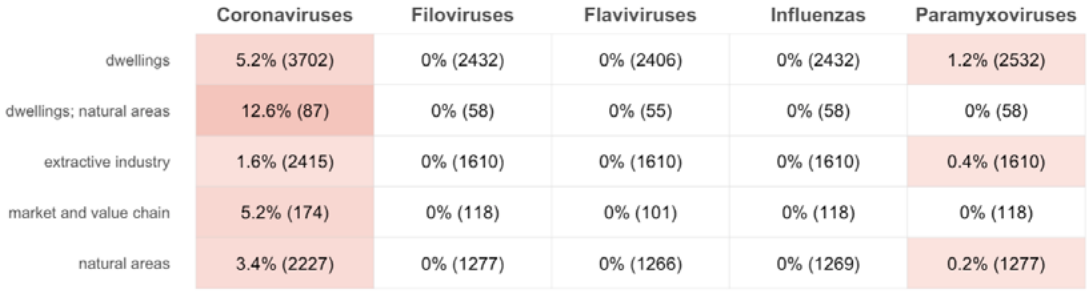
VIRUS TABLE

				# OF POSITIVE INDIVIDUALS		
VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Loei Hospital	1	0	1
	Human coronavirus HKU1	Human	Ratchaburi	1	0	1
	PREDICT_CoV-16	Indian Flying Fox, Lyle's Flying Fox	Chonburi	34	31	3
	PREDICT_CoV-17	Indian Flying Fox, Lyle's Flying Fox	Chonburi	88	77	11
	PREDICT_CoV-22	Dawn Bat	Khao Chakan	7	7	0
	PREDICT_CoV-24	Greater Short-Nosed Fruit Bat	Loei	1	0	1
	PREDICT_CoV-27	Large Asian Roundleaf Bat	Khao Chakan	3	3	0
	PREDICT_CoV-41	Wrinkle-Lipped Free-Tailed Bat	Ratchaburi	1	1	0
	PREDICT_CoV-47	Wrinkle-Lipped Free-Tailed Bat	Khao Chakan, Loei, Ratchaburi	19	5	14
	PREDICT_CoV-56	Greater Short-Nosed Fruit Bat	Loei	1	0	1
	PREDICT_CoV-68	Indian Flying Fox, Lyle's Flying Fox	Chonburi	13	13	0
	PREDICT_CoV-82	Wrinkle-Lipped Free-Tailed Bat	Khao Chakan, Loei, Ratchaburi	42	40	2
	PREDICT_CoV-99	Wrinkle-Lipped Free-Tailed Bat	Ratchaburi	16	2	14
	Bat coronavirus 1	Schreiber's Long-Fingered Bat, Western Long-Fingered Bat	Khao Chakan, Loei	3	3	0
	Bat coronavirus HKU10	Large Asian Roundleaf Bat	Khao Chakan	4	4	0
	Porcine epidemic diarrhea virus	Horsfield's Myotis	Khao Chakan	1	1	0
	Rousettus bat coronavirus GCCDC1/346/356	Dawn Bat	Loei	1	1	0
	Longquan Aa mouse coronavirus	Bower's Rat	Loei	1	0	1
	Murine coronavirus	Cook's Mouse, Fawn-Coloured Mouse, Indomalayan Niviventer, Neill's Long-Tailed Giant Rat, Oriental House Rat, Polynesian Rat, Red Spiny Rat	Loei, Ratchaburi	36	28	8
	Betacoronavirus 1 (Bovine CoV)	Domestic Cow	Ratchaburi	1	1	0
Paramyxovirus	Human parainfluenzavirus 1	Human	Loei Hospital	8	2	6
	Measles virus	Human	Loei Hospital	1	0	1
	PREDICT_PMV-2	Lyle's Flying Fox	Chonburi	4	4	0
	PREDICT_PMV-3	Lyle's Flying Fox	Chonburi	2	1	1
	PREDICT_PMV-4	Indian Flying Fox	Chonburi	1	0	1
	PREDICT_PMV-5	Lyle's Flying Fox	Chonburi	5	4	1
	PREDICT_PMV-6	Lyle's Flying Fox	Chonburi	4	3	1
	PREDICT_PMV-10	Indian Flying Fox, Lyle's Flying Fox	Chonburi	2	0	2
	PREDICT_PMV-85	Lyle's Flying Fox	Chonburi	1	1	0
	PREDICT_PMV-125	Indian Flying Fox	Chonburi	1	1	0
	PREDICT_PMV-128	Lyle's Flying Fox	Chonburi	2	2	0
	PREDICT_PMV-140	Lyle's Flying Fox	Chonburi	1	1	0
	PREDICT_PMV-142	Lyle's Flying Fox	Chonburi	1	0	1
	PREDICT_PMV-155	Wrinkle-Lipped Free-Tailed Bat	Loei, Ratchaburi	9	9	0
	Nipah virus	Lyle's Flying Fox	Chonburi	6	6	0
	PREDICT_PMV-20	Polynesian Rat	Loei	2	0	2
	PREDICT_PMV-86	Lesser Ricefield Rat, Polynesian Rat	Loei	3	3	0
	PREDICT_PMV-159	Fawn-Coloured Mouse	Loei	1	1	0
Influenza virus	Influenza A	Human	Loei Hospital	38	32	6
	Influenza B	Human	Loei Hospital	10	7	3
Flavivirus	Dengue virus serotype 2	Human	Loei Hospital	1	1	0
	Zika virus	Human	Chulalongkorn Hospital	1	1	0
Picornavirus	Human enterovirus B	Human	Loei Hospital	2	0	2
Total				**380**	**296**	**84**

EPIDEMIOLOGICAL & BEHAVIORAL RISK

The PREDICT team assessed zoonotic disease risk using a multi-pronged approach, which included analysis of ecological wildlife-human interfaces, epidemiological risk based on virus detection data in wildlife, scenario and spatial modeling, and quantitative analysis of behavioral survey data.

The PREDICT project's risk-based approach to surveillance included targeted wildlife sampling from key animal-human interfaces at each sampling site. Overall, the highest rate of virus detections at these disease interfaces for coronaviruses and paramyxoviruses in animals occurred around human dwellings. A high percentage of coronavirus positive individuals were also sampled in the market value chain, although with more limited sampling in this setting. While the total number of coronavirus detections was relatively low in the extractive industry setting, including bat guano mining from Ratchaburi Province, this interface was important given the high level of exposure to animal excreta and the types of viruses discovered (MERS-related CoVs).



	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
dwellings	5.2% (3702)	0% (2432)	0% (2406)	0% (2432)	1.2% (2532)
dwellings; natural areas	12.6% (87)	0% (58)	0% (55)	0% (58)	0% (58)
extractive industry	1.6% (2415)	0% (1610)	0% (1610)	0% (1610)	0.4% (1610)
market and value chain	5.2% (174)	0% (118)	0% (101)	0% (118)	0% (118)
natural areas	3.4% (2227)	0% (1277)	0% (1266)	0% (1269)	0.2% (1277)

FIGURE 5. ***Heatmap summarizing sampling effort and detection of positives in wildlife for the five priority virus groups by disease risk interfaces (e.g. market value chain, wildlife near human dwellings, etc.) at the sampling sites. Dwellings include rural, human inhabited sites in located in close proximity to wildlife populations, in some cases those sites geographically overlap with uninhabited natural areas.***

MAPS & MODELS FOR THE THAILAND VIROME PROJECT PARTNERSHIPS

To further assess risk from the PREDICT project's surveillance data and virus findings, the PREDICT Thailand team, together with global PREDICT partners, combined virus detection data with epidemiological and ecological risk analyses. Analytical reports were generated directly from the EIDITH database and were shared with country teams throughout the project to help them better understand country and regional risk and contextualize their findings as new data came in. These analyses included geographic distribution of detected viruses, host range, virus discovery rates, comparison of risk factors across sites in a country, and prioritization of species and sites for future surveillance activities (e.g. for the Thailand Virome Project Partnership (TVPP)).

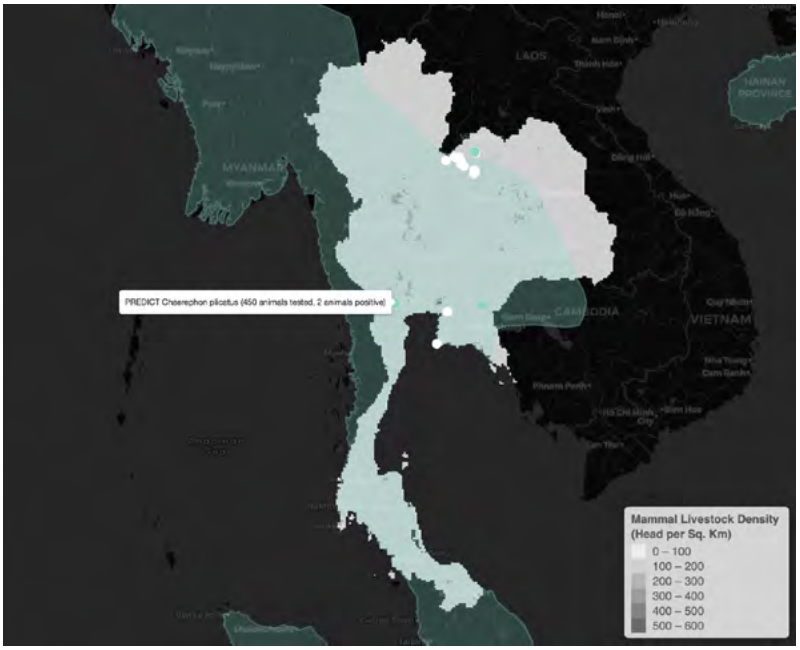
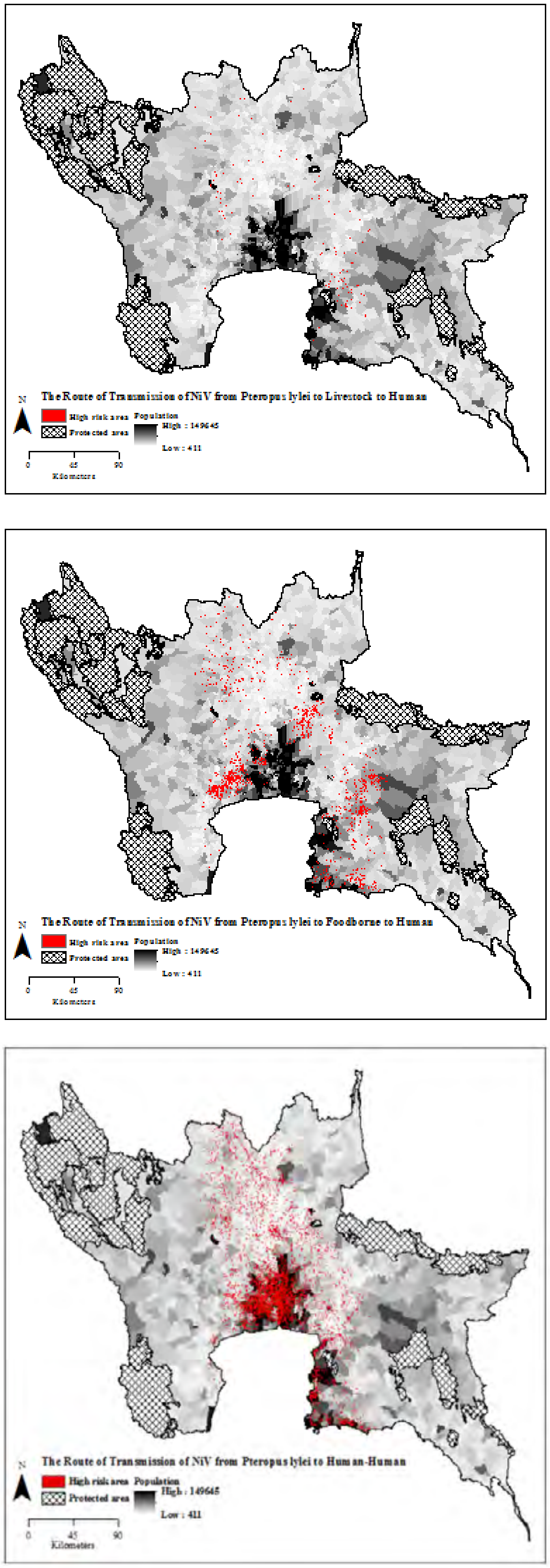


FIGURE 6. ***Output from the PREDICT Thailand Modeling & Analytics report showing virus data in a geographic context to explore risk assessments. Data shown here are for a novel coronavirus, PREDICT_CoV_47, with the known host species geographic range highlighted (green area) and sampling site data from PREDICT Thailand as white dots (PREDICT CoV_47 not detected) and green dots (virus was detected). Data overlaid with global data on mammal livestock density, grey-scale base layer (FAO Gridded Livestock of the World).***

To further assess epidemiological risk from specific wildlife virus findings, we analyzed virus detection data for every virus detected by the PREDICT project in Thailand at least 30 times against various host, sampling, and ecological traits. Using a multi-variable Least Absolute Shrinkage and Selection Operator (Lasso) regression analysis, we were able to identify the most significant variables that predict whether or not a given virus would be detected. PREDICT Thailand identified two newly discovered coronaviruses (with an unknown ability to infect people), PREDICT_CoV-17 and PREDICT_CoV-82, from 89 and 42 different animals, respectively. PREDICT_CoV-17 was found frequently in *Pteropus* spp. (large fruit bats) from Chonburi Province (Fig. 7), and PREDICT_CoV-82 was found in *Chaerephon plicatus* (cave roosting insectivorous bat) from Ratchaburi Province. Interestingly, the most important predictor for detecting both of these viruses was sampling juvenile bats. This finding, that the age structure of wildlife populations is an important risk factor, was also noted for coronaviruses in East Africa (Montecino-Latorre et al., 2020), and in global analyses for other viruses.



Nipah virus represents a clear and present threat, as it has been discovered to be circulating widely in bat populations in Thailand but has not yet been found in pig or human populations that interact with bat populations. The PREDICT team supported critical investigations into the natural ecology of Nipah virus in bats, and the risk posed to local communities living in close contact with bat populations. As part of the effort to further strengthen the capacity for zoonotic disease risk modeling in Thailand, Ms. Aingorn "Na" Chaiyes, a PhD student and member of the PREDICT team in Thailand, completed a Modeling & Analytics fellowship with EcoHealth Alliance in New York for 5 weeks in 2016. Ms. Chaiyes' PhD research focused on using ecological niche modeling and spatial analyses tools to better understand the risk of Nipah virus spillover across Thailand. This research resulted in two peer-reviewed publications, one published and one currently in review.

FIGURE 8. *Analysis of Nipah virus spillover risk in Thailand using habitat suitability models of the primary bat host,* **Pteropus lylei**, *and spatial data for known drivers of viral spillover (e.g. pig density and presence of fruit orchards). Showing areas with a high risk of Nipah viral transmission from* **P. lylei** *to livestock (A), via foodborne (fruit orchard) sources (B), and directly to humans (C) in Thailand.*

FIGURE 7. *Heatplot of PREDICT_CoV-17 prevalence by sampling season and sex of bats sampled. PREDICT_CoV-17 was detected 89 times in* **Pteropus spp.** *(flying foxes) from Chonburi Province. No differences in prevalence were observed between male and female bats, but significantly higher prevalence was detected in wet season vs. dry season, which is likely related to the abundance of juvenile bats.*

IN-DEPTH BEHAVIORAL RISK INTERVIEWS

In Thailand, behavioral risk questionnaires were administered to community members enrolled in our community virus surveillance. This included 243 participants from the concurrent (wildlife, people, domestic animals) sampling site in Chonburi Province, and 230 participants from the concurrent sampling site in Ratchaburi Province. In addition, 200 participants from Loei Province enrolled in the clinic-based surveillance were also surveyed. Some key differences in each setting were observed with implications for the frequency of animal contacts and associated risk of exposure to zoonotic pathogens, as well as other differences such as hygiene practices, health-seeking behavior, and knowledge about zoonotic risk.

In Loei Province, a more rural setting bordering mountainous areas, there was a high degree of contact with live animals in and around the home, including with rodents, and 39.5% of participants reported eating undercooked or raw meat and organs from animals. We also analyzed the specific types of contact with each wildlife taxonomic group (bats, rodents, and primates) from each site. For example, in Loei Province, 27% of participants noted rodents around their homes and 8% have slaughtered rodents for food (Figure 9). In Ratchaburi Province, relatively few individuals have knowingly or directly interacted with bats, but a few individuals did acknowledge being bitten or scratched recently. These behaviors overlap in an area where we also detected a variety of rodent-borne coronaviruses and paramyxoviruses but did not find evidence of human infection with these viruses.

There appeared to be a lack of general consensus on whether live animal markets could be cause for concern, with respondents in Chonburi and Loei being split evenly on the risk that these sites presented (with 49% and 52%, respectively, indicating that "Yes," they were worried about diseases in live animals in markets), and respondents in Ratchaburi being just slightly more concerned than not (58%). Knowledge of the risk of zoonotic disease was somewhat limited, with between 18-46% of participants (depending on site) acknowledging that slaughtering or butchering animals with an open wound could lead to disease infection. 21% and 33% of respondents in Chonburi and Loei, respectively, indicated that they did not know if there were associated dangers in this case, and 66% of respondents in Ratchaburi reported that there would be nothing to worry about. These responses, which acknowledge uncertainty and indicate misinformation, suggest potential entry points for targeted public health messaging and behavior change communication programming – particularly for those whose occupational or cultural activities center around live markets.

The opportunity in Thailand to share new information and challenge existing beliefs is further reinforced when looking at the responses among those who had reported an unusual illness in the last year: the majority of respondents across each site admitted that they did not know the origins of their illness. Together, our preliminary findings suggest that some communities, including ones with high levels of animal contact, would make good targets for continued community engagement and education campaigns about zoonotic disease transmission.

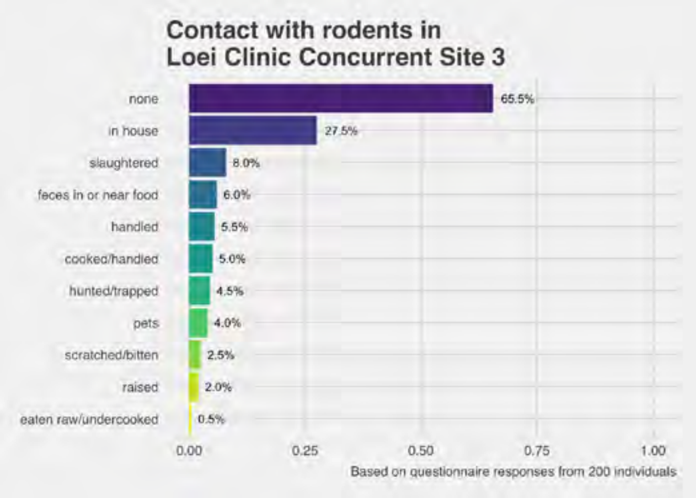


FIGURE 9. ***Reported human contact with rodents at various interfaces by participants from Loei Clinic.***

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

The PREDICT team provided summaries of surveillance and laboratory findings to the communities involved in the project. The team also shared resources and strategies with community partners designed to reduce risks for viral transmission while balancing health and conservation goals. In Ratchaburi Province, the PREDICT team conducted community outreach campaigns targeting the bat guano miner community to increase awareness and promote risk reduction strategies. The PREDICT-developed risk reduction and behavior change communication resource *Living Safely with Bats* was translated into Thai language and used to highlight ways that communities can live safely and humanely in concert with bats. More than 100 participants learned about bats, their important contributions to ecosystem services, and strategies that could reduce the risk of spillover, amplification, and spread of novel viruses. The PREDICT team also organized a "Community One Health Meeting" for villagers within Panatnikhom District, Chonburi Province on November 28, 2018 to educate them on the zoonotic diseases carried by bats and dogs. Other examples of community engagement include organizing the "Global One Health Day" in 2017 and 2018 with government partners from 7 One Health organizations and the "Disease X Conference" to disseminate research findings to the academic and layman communities, held in Bangkok on December 17, 2018.

CAPACITY STRENGTHENING

The PREDICT team was host to a large number of One Health trainings and collaborative workshops aimed at increasing capacity for preemptive disease surveillance and detection across the country. These trainings ranged from field, laboratory topics, and policy level collaborations with government as well as researchers and health professionals.

The PREDICT team conducted biosafety training in bat and rodent sampling for >50 local government staff from the Department of National Parks (DNP) who worked alongside PREDICT project staff in Loei and Ratchaburi Provinces in July 2016. The team also hosted two SEAOHUN (EPT One Health Workforce) fellows from Myanmar and Malaysia. Both individuals received training in biosafety and bat sampling.

The PREDICT project in Thailand made significant contributions to improving the capabilities of the national laboratory system in-country and in the greater Asia region by hosting workshops with FAO partners in virus detection methods and through numerous in-country events. In 2017, the team participated in the "Joint External Evaluation of IHR Core Capacity in Thailand" meeting held at the MOPH. PREDICT project protocols for broad virus screening at a family level were suggested as an alternative method to detect viruses with zoonotic potential to the committee, and examples were given to show how these new tools have been implemented in Thailand at Chulalongkorn and DLD laboratories.

PREDICT collaborated with the Department of Disease Control, MOPH to strengthen influenza surveillance and reporting. These efforts were specifically directed towards improving the national influenza reporting system by strengthening the capacity of diagnostic laboratories at the regional level. The PREDICT team organized a hands-on training workshop, sponsored by the National Science and Technology Development Agency of Thailand, on real-time PCR diagnostics for influenza virus infection from human specimens. The workshop was organized for 14 laboratories from the Department of Disease Control in March 2017. Following the training, the PREDICT team participated in a meeting organized by the Department of Disease Control and provided expert advice on database development for influenza surveillance in Thailand.

The PREDICT project in Thailand, together with PREDICT global partners, conducted training workshops for staff from Loei hospital and the Ratchaburi Office of Disease Prevention and Control on quantitative and qualitative methods for behavioral risk surveillance and intervention development. The multi-day training involved hands-on exercises in behavioral survey collection and qualitative research, and ethical considerations for human subject research.

Bat carcasses were sampled by the PREDICT team in Nam's cave, Kanchanaburi Province, during an investigation into the cause of death of over 1,000 bats with partners at the Department of National Parks

PHOTO: PREDICT/THAILAND

OUTBREAK PREPAREDNESS & RESPONSE

Due to its abundant experience diagnosing emerging viruses over the past 10 years, PREDICT Thailand's laboratory has been designated as the Ministry of Public Health's reference laboratory for identification of EIDs. At the request of the Thai Ministry of Public Health (MOPH), the PREDICT team provided technical assistance for virus detection during several noteworthy infectious disease investigations. The first imported MERS case in Thailand, in 2015, was identified and confirmed by the PREDICT lab team within 24 hours after receiving the specimen. Contact tracing was implemented immediately, and known contacts were placed in self-isolation for 14 days, including air-crew, and no outbreak occurred. During the Ebola outbreak in Africa in 2014, a PREDICT protocol was used to test human samples from suspected imported cases in Thailand during the first few months when the standard diagnostic assay was not available in-country. In January 2020, the PREDICT team also detected the first human COVID-19 case in-country (see Special Features below).

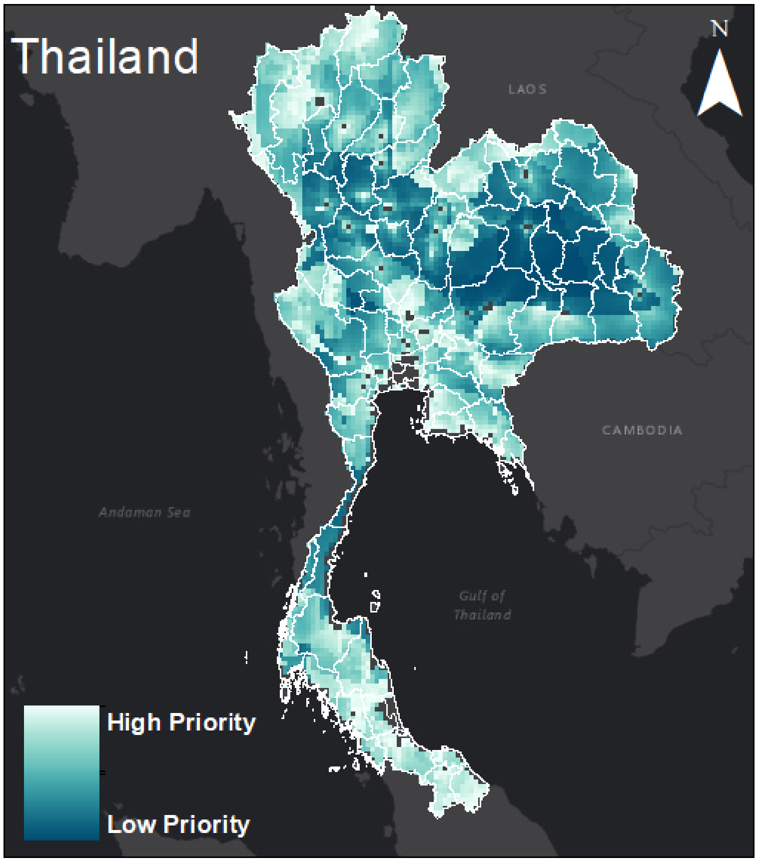
In partnership with the government Department of National Parks, Wildlife and Plant Conservation (DNP), the PREDICT team also investigated the cause of death of more than 1,000 bats in Kanchanaburi Province in November 2015. As part of the investigation, the team sampled more than 53 bats in the field and tested the samples for the presence of viral RNA from six virus groups at the project laboratory including coronaviruses, paramyxoviruses, rhabdoviruses, filoviruses, lyssa viruses, and influenza viruses (additional funding for laboratory assays was provided by FAO). Seven coronaviruses in fecal samples and four paramyxoviruses in urine specimens were identified; these results were confirmed by genetic sequencing. There is no evidence at this time to suggest these viruses pose a threat to human health or caused the bat die-off. All results were reported to government partners as part of the surveillance and outbreak investigation. It was concluded that the mortality event was likely due to weather (flooding within the cave) and not a viral pathogen.

ADVOCATING FOR A GLOBAL VIROME PROJECT

On January 31, 2017, the PREDICT team hosted a Global Virome Project (GVP) seminar at Chulalongkorn University Hospital. The seminar was part of broader efforts to garner interest in the GVP and Emerging Pandemic Threats (EPT) projects, as well as zoonotic pathogens and emerging infectious disease in Thailand. On January 29, 2018, during the Prince Mahidol Award Conference (PMAC) in Bangkok, Thailand, the PREDICT Project and the Global Virome Project (GVP) planned and successfully hosted a meeting titled "Introducing the Global Virome Project". This was the second global in-person GVP meeting following from the kick-off meeting in Beijing, China.

To set the stage for GVP, the PREDICT project contributed to the predicted virus diversity and costs of virus discovery analyses, recently published in Science (Carroll et al., 2018). Building on this work, the PREDICT team developed a spatial modeling approach to identify priority sites for optimal virus sampling in wildlife at a 10 x 10 km resolution in countries around the world.

The first country with a completed, new GVP site selection analysis was Thailand, in preparation for a Thailand National Virome Project (also known as the Thailand Virome Project Partnership – TVPP) workshop held in Bangkok in October 2018, where the findings were presented to Thai government stakeholders. The TVPP is currently developing its research activities and is in a pilot program phase under guidance from the wildlife sector at the Department of National Parks (DNP). At present, DNP is collaborating with the PREDICT Thailand team to conduct virus testing from wildlife specimens as a pilot for the TVPP at high priority locations.



Future sampling priority areas in Thailand, ranked from most optimal (white) to least optimal (dark blue). Data analysis was conducted for the Thailand Virome Project Partnership planning meetings, including the development of interactive scalable maps. We used the Zonation Conservation Planning Software to select and rank sites of highest sampling value based on the number of unique zoonotic viruses predicted to occupy each grid cell (derived from Olival et al., 2017) and the relative cost of accessing each site from nearby cities of greater than 50,000 people (Weiss et al., 2018). We identified optimal areas for virus sampling using a decision support system for spatial planning that allocates resources (e.g. sampling costs) and prioritizes areas. The result is a ranking of all potential sampling sites in Thailand based on the highest return on investment for sampling in a particular area.

SPECIAL FEATURES

THAM LUANG CAVE RESCUE

The PREDICT team played a key role during the rescue of the boys trapped in the Tham Luang cave in July 2018, which was a multi-national and collaborative effort in the global spotlight. PREDICT supported the Thai Ministry of Public Health in testing samples from the 13 survivors for diseases. Being trapped in a cave without any access to food or clean water might have compromised the immune system of the boys, increasing their susceptibility to bacterial, fungal, viral or other infections including potential bat, rodent, or insect-transmitted pathogens from the environment. PREDICT PCR diagnostic protocols were used to broadly screen for known and unknown viruses, and testing results were shared in real-time with a network of public health laboratories in Thailand. Fortunately, none of the rescued young soccer players tested positive for any pathogens related to their time in the cave.

On June 23, 2018, twelve boys from the Wild Boars soccer team and their coach set out on an adventure to explore the vast Tham Luang Nang Non cave complex ("The Great Cave of the Sleeping Lady"), near the village of Pong Pa in northern Thailand. But due to the rapid onset of monsoon rains, caverns in the cave began to flood and the team became trapped in deep caverns, separated from the entrance by miles of flooded chambers. The boys were trapped and missing for nine days before specialist rescue divers located them. Then another nine days passed before all 12 boys and their coach were rescued from the cave by the divers in a massive operation that involved more than 10,000 people, miles of hose and rope, pumps, generators, and countless supporters. The collaborative rescue was a multi-national and interdisciplinary success, and even featured One Health expertise, as the PREDICT partner laboratory at Chulalongkorn University was requested by the Thai government and the Ministry of Public Health (MOPH) to provide technical assistance with disease screening following the team's rescue.

Following the rescue, as the soccer team members were carefully monitored by health experts, PREDICT's lab team provided advice to MOPH staff on safe collection of specimens for virus screening, and then prepared to test the specimens for infectious pathogens. The PREDICT project's sampling protocols were used to collect the samples and our project's virus detection protocols, which can identify both known and emerging viral threats, were used for screening. In addition to testing at our PREDICT lab, specimens were also shared with One Health network laboratories, including the Department of Medical Sciences, Thai and US Armed Forces Research Institute of Medical Sciences, and the Department of Microbiology and Parasitology, Faculty of Medicine, Chulalongkorn University. Lab teams simultaneously ran specific assays for targeted diseases along with the PREDICT project's family-wide virus detection assays to ensure results within 24 hours.

The exhaustive disease screening following the cave rescue demonstrates Thailand's proactive approach to surveillance and response, especially for emerging diseases and disease risks from bats and caves. In addition, the strengths of the national laboratory system were prominent, as Thailand's disease detection network successfully collaborated to share data and information for rapid detection and identification of disease threats.

SPECIAL FEATURES

MERS-CoV PREPAREDNESS & EARLY DETECTION

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is an emerging infectious disease that was first detected in 2012. MERS-CoV is thought to have an animal source with an ancestral origin in bats and contemporary transmission from camels to people in the Middle East. Infection may also be transmitted between humans through airborne spread or direct contact, and several outbreaks have occurred around the world from imported human cases. In June 2015, Thailand saw its first case of MERS-CoV, brought into the country by an international traveler.

As a result of prior preparedness efforts, including virus discovery for human infections as part of the PREDICT project, training on sampling for potential MERS-CoV infections, and MERS-CoV laboratory screening protocols, the country had strong capacities in place. Paired with infection control practices, intensive surveillance was rapidly implemented (e.g. temperature checks) in high-risk settings including points of entry into the country and in healthcare settings. Suspected MERS-CoV patient samples were rapidly tested (only seven hours for first results and 24 hours for confirmation) at the WHO Collaborating Center for Research and Training on Viral Zoonoses at Thailand's Chulalongkorn University (which reports to the Ministry of Health). The PREDICT team tested hundreds of potential contacts identified via contact tracing by the Ministry of Public Health from several separate MERS-CoV introductions. No secondary infections were detected in Thailand suggesting no human to human transmission occurred.

Given the limited knowledge on MERS-CoV when it first emerged, the PREDICT team leveraged its ongoing wildlife surveillance efforts with One Health partners to continue to test for coronaviruses in wildlife and domestic animals in the country to help improve understanding about this group of viruses. At the request of the Government of Thailand, the PREDICT team also hosted a training on how to safely sample camels for MERS-CoV detection in February 2014. Surprisingly, there are over 50 camels in Thailand, located at various zoos and other captive facilities, and officials wanted to be sure there was no evidence for MERS-CoV infection in these captive animals. The Department of Livestock and Development followed up after the training with sampling and testing of camels in the country.

SPECIAL FEATURES

COVID-19 DETECTION IN THAILAND

The COVID-19 infection was first confirmed at the PREDICT lab at Chulalongkorn Hospital and PREDICT project virus group diagnostic protocols were used to detect and characterize the novel coronavirus from a Wuhan tourist before the specific testing protocol from WHO was available. The finding and sequence confirmation (best matched to bat SARS-related coronavirus, accessed to GenBank on January 9, 2020) was reported to MOPH within 24 hours after receiving the specimen. On January 11, 2020 the sequence was re-analyzed with the first whole genome sequence of SARS-CoV-2 from China, and the two viruses were found to be identical for a short region of the genome. The whole genome was then characterized using next-generation sequencing technology and was found to be >99% identical to SARS-CoV-2 from Wuhan.

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PREDICT VIET NAM

ONE HEALTH IN ACTION (2009-2020)

Preventing pandemics, protecting global health

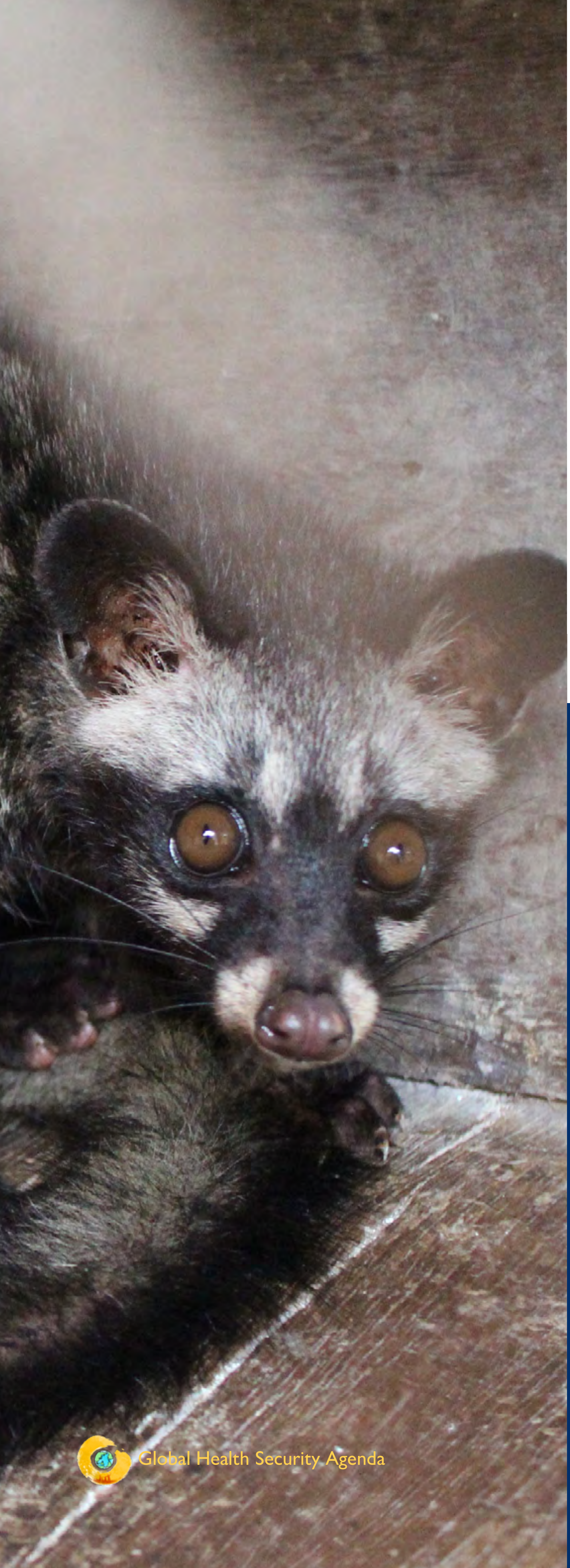
The PREDICT project in Viet Nam was a collaborative effort with the Vietnamese government agencies within the environment, animal health, and public health sectors to address the threat of emerging pandemic diseases facilitated by the interaction of wildlife, domestic animals, and humans (the animal-human interface). The PREDICT team focused on investigating and understanding the potential transmission of infectious diseases between wildlife, livestock, and humans at key human/wildlife/domestic animal interfaces along the animal value chains and animal production systems, including the wildlife trade, live animal markets, and bat guano collection sites to prevent pandemic disease emergence and negative impacts on human health. The PREDICT team also conducted behavioral surveillance to gather relevant information about risky human behavior and practices to provide a better understanding of the drivers for zoonotic spillover and host-pathogen dynamics. While core PREDICT objectives centered on protecting human health, wildlife conservation benefits included improved diagnostic laboratory capacity for wildlife disease surveillance and greater governmental awareness and investment in wildlife population management.

The PREDICT project used a One Health approach to understand the dynamics of zoonotic virus evolution, spillover, amplification, and spread to inform prevention and control. Samples were safely collected at the high-risk interfaces from wild rodents, bats, carnivores, and non-human primates, in addition to human populations. Through this collaborative effort with Vietnamese research, academic, and government institutions, the PREDICT team collected nearly 7,000 samples from wildlife and completed over 16,000 assays in Vietnamese and international laboratories to identify known and novel viruses.

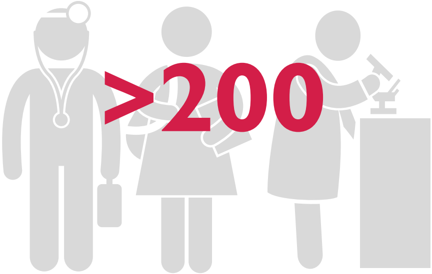
The PREDICT project's zoonotic disease surveillance was strategically designed to train, equip, and enable surveillance personnel from the animal and human health sectors to collect data and build the evidence base for both priority zoonoses and emerging and re-emerging diseases such as viral hemorrhagic fevers in vulnerable and high-risk areas. Shared animal and human surveillance data and findings helped catalyze formal information sharing between animal and human surveillance systems. In addition, our surveillance engaged local communities in high-risk areas for disease transmission and emergence and fostered improved recognition of zoonotic diseases and awareness of transmission pathways and prevention and control options.

LOCAL PARTNERS

- Department of Animal Health (DAH), Ministry of Agriculture and Rural Development (MARD)
- Dong Nai Province Forest Protection Department
- Endangered Primate Rescue Center (EPRC)
- Food and Agriculture Organization (FAO)
- National Institute of Hygiene and Epidemiology (NIHE), Ministry of Health (MoH)
- Regional Animal Health Office No. 6 & 7 (RAHO6&7)
- Save Vietnam's Wildlife (SVW)
- University of California, Davis (UCD)
- USAID
- Viet Nam National University of Agriculture (VNUA)
- Viet Nam One Health University Network (VOHUN)
- Wildlife Conservation Society (WCS)
- World Health Organization (WHO)

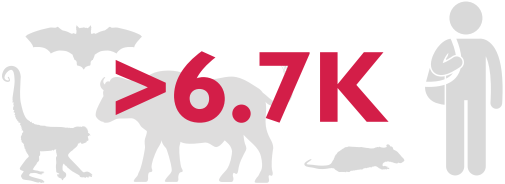


Global Health Security Agenda



>200

DEVELOPED the One Health Workforce by training more than 200 people in Viet Nam.

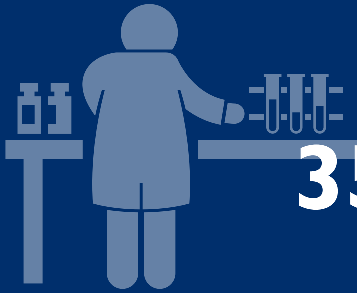


>6.7K

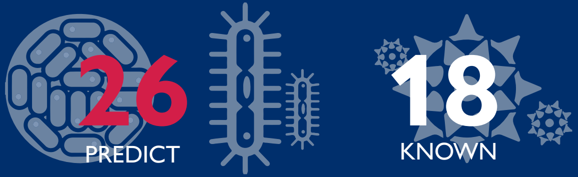
OPERATIONALIZED One Health surveillance and sampled over 6.7K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Viet Nam National University of Agriculture
- Regional Animal Health Office No. 6
- National Institute of Hygiene & Epidemiology



35,370
TESTS



26
PREDICT

18
KNOWN

DETECTED 44 unique viruses in both animals and human populations.

NGUYEN THI THANH NGA

Health Team Manager, *Wildlife Conservation Society*

"I started my career as a member of the PREDICT project. Being a part of the team helps me deepen my expertise, improves my skills, and gives me a golden chance to explore the areas that I'm interested in – wildlife, wildlife health and zoonotic diseases. PREDICT has helped me enhance my veterinary skills and its findings bring value to animal health, especially wildlife health, in Viet Nam."

VU TRONG DUOC

Human Health Surveillance & Laboratory Lead, *National Institute of Hygiene & Epidemiology*

"The PREDICT project has built the capacity for government staff directly involved in the project to improve their existing professional skills and update other knowledge from the project. With the National Institute of Hygiene and Epidemiology, following the completion of the project, the results provided significant contributions to develop a pathogen detection and monitoring plan, and help us to confidently continue other studies based on the experience gained from the PREDICT project."

ACHIEVEMENTS

- We established a working relationship with public health sectors and collaborated with animal health sectors to implement a One Health project.
- More than 140 project partners were engaged in building capacity for novel virus detection and concurrent surveillance for zoonotic diseases in human and animal populations in Viet Nam through PREDICT. Specifically, the skills-training in wildlife disease surveillance has built core capacity within Viet Nam's human and animal health sector to investigate and characterize the disease risks associated with the unregulated and illegal trade in wildlife.
- PREDICT Viet Nam strengthened diagnostic capacity in national animal health and public health laboratories for the early detection of known and novel viruses of pandemic potential to predict and prevent disease emergence.
- PREDICT Viet Nam's human syndromic surveillance was conducted in three sites across Viet Nam with 1,200 people enrolled in the surveillance.
- PREDICT Viet Nam worked in close collaboration with the Secretariat Office of the Viet Nam One Health Partnership, General Department of Preventive Medicine of Ministry of Health, Department of Animal Health of Ministry of Agriculture and Rural Development, Food and Agriculture Organization of the United Nations (FAO), and the World Health Organization (WHO) to improve coordination of surveillance efforts across the wildlife, livestock, and human health sectors for influenza and other viruses with pandemic potential in populations in Viet Nam. PREDICT's One Health approach to zoonotic disease surveillance was linked with national surveillance systems for severe acute respiratory illness (SARI) in people, and influenza surveillance in livestock, so that data can be analyzed jointly to inform disease risk assessments and support disease prevention strategies designed to disrupt the amplification and spread of important respiratory pathogens. This coordination of surveillance activities in Viet Nam is also known as Longitudinal Influenza Surveillance Network (LISN) which is conducted in livestock, wildlife and humans at their interface or points of contact to identify influenza A virus and other potential pandemic threats.

ONE HEALTH SURVEILLANCE

Prioritizing & operationalizing risk reduction

Viet Nam has been a leader in advancing One Health in practice since 2006 through development of coordinated approaches to tackle Highly Pathogenic Avian Influenza and in its current role as a co-lead for the Global Health Security Agenda (GHSA) Zoonotic Diseases Action Package. In 2016 the country established an updated 5-year One Health Strategic Plan for zoonotic diseases, which integrates One Health across more than 20 national sectoral and topical plans to strengthen capacity, enhance preparedness, and reduce impact of priority zoonoses and antimicrobial resistance. The plan's six main focus areas include a dedicated item on prevention of infectious disease emergence (Focus Area 3: One Health approaches for managing zoonotic agents with pandemic potential that are yet to emerge), with specific outcome indicators on: i) Identification of risk factors or interfaces associated with spillover, amplification and/or spread, and ii) Implementation of risk reduction strategies based on identified risk factors. The plan also recognizes the need for environmental health research on disease drivers (for example, on land use practices) and greater engagement overall of the environment sector through guidelines, policies, and resource allocation to support One Health capacity building.

WHAT ONE HEALTH ADDED

To date, the emphasis for novel disease outbreaks has been on mobilizing effective and rapid response to avoid international disease spread. Viet Nam's application of One Health promotes a preventive stance that moves upstream to target pathogen spillover at the source to avoid or reduce human and animal disease and their associated health and economic consequences. The plan has been made operational through initial activities at key interfaces where wildlife-human exposure is common and presents potential zoonotic disease transmission pathways (e.g., live animal markets, bat guano collection sites, and wildlife restaurants). PREDICT has been a key partner in strengthening capacity for sample collection and screening for novel pathogens, addressing key challenges identified in the plan, including workforce development for risk monitoring and mitigation at high-risk animal-human interfaces (wildlife farms, bat guano harvest sites, and rodent trade routes) through on-the-job training for Dong Nai Province Forest Protection Department, the Regional Animal Health Office No. 6 & 7, and National Institute of Hygiene and Epidemiology staff who participated in PREDICT surveillance planning, sample collection, and diagnostic activities. With PREDICT's support, all partner laboratories, including national public health and animal health laboratories in Viet Nam, are trained in the full range of activities required for safely detecting zoonotic viruses, such as biosafety and biosecurity, cold chain, safe sample storage, data management, safe sample transport and shipping, and molecular virus detection techniques.

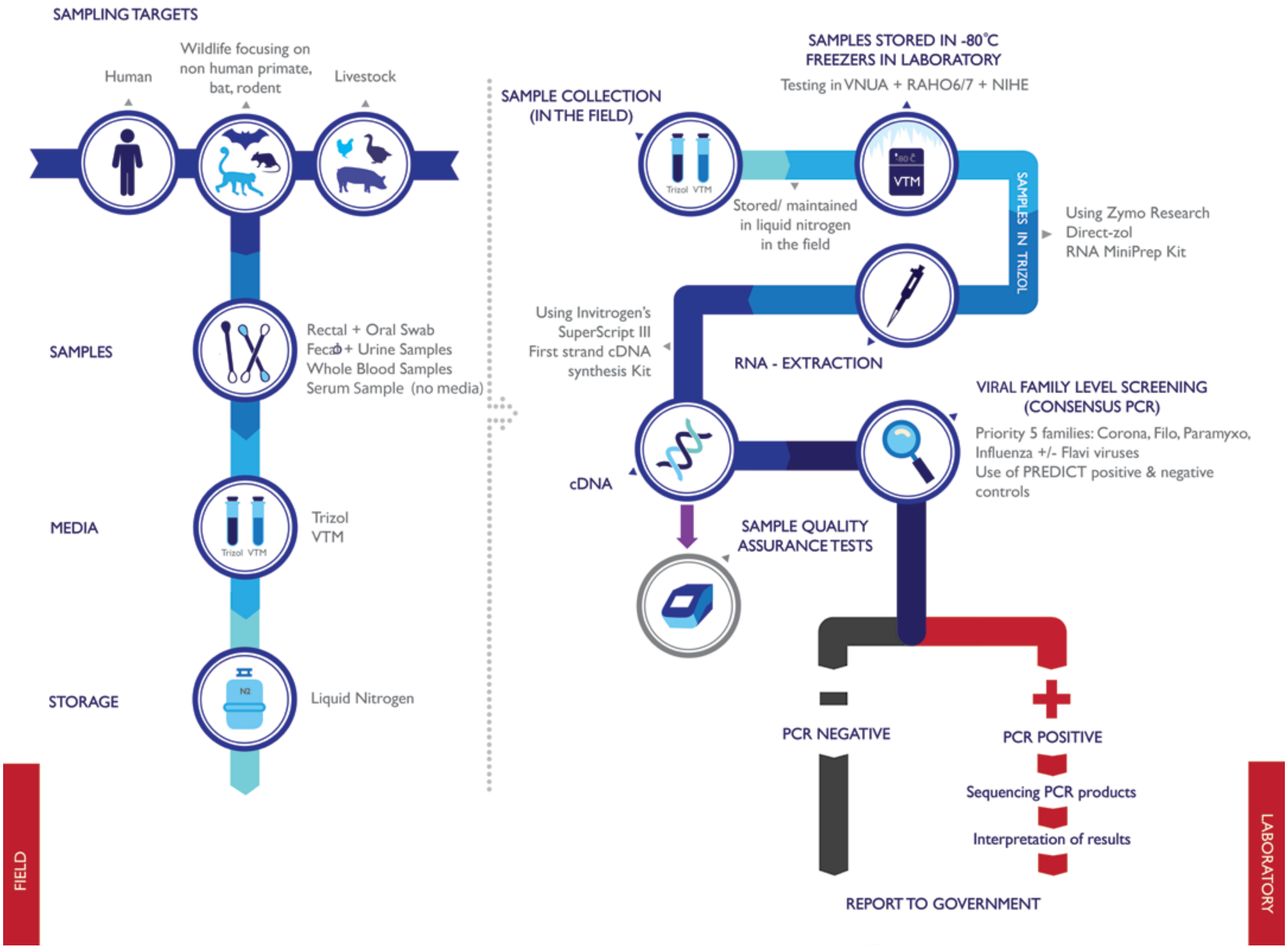
The country has also recognized the need for multisectoral information sharing to promote biodiversity and health mainstreaming across a range of stakeholders. For example, PREDICT shared results and lessons on human health risks associated with the illegal wildlife trade at a United Nations Office on Drugs and Crime (UNODC) training on the Penal Code in July 2018, helping to strengthen awareness about a potential driver of disease emergence in addition to biodiversity loss. Viet Nam's national commitment to reducing the threat of novel pathogens is a model for all countries to get ahead of disease emergence events, which is adaptable to high-risk species and exposure interfaces relevant to each country or community.

Read more at **bit.ly/2BKgrxg**

VIRUS DETECTION

PREDICT is the first project to use consensus polymerase chain reaction (cPCR) as the primary method for detection of both known and novel viruses in a wide range of samples and host species on a global scale (Goldstein et al., 2013; Anthony et al., 2013). The advantages of this approach include:

- An inexpensive testing method (cPCR) run on basic equipment, such as thermal cyclers for conventional PCR already available in animal and human health laboratories in Viet Nam.
- The "universal" amplification of viruses within a given virus group or genus.
- Synthetic 'universal controls' that provide standardized control material without any danger of pathogen transmission.
- Increased safety to laboratory workers as any microbes in the samples are killed during the PREDICT nucleic acid extraction steps so laboratory workers are not exposed to dangerous pathogens.
- Ability to extend the PREDICT diagnostic strategy beyond the detection of viruses in wildlife to the diagnosis of mystery illnesses in medical hospitals or in solving an unknown disease outbreak in livestock or domestic animals.



VIRUS TABLE

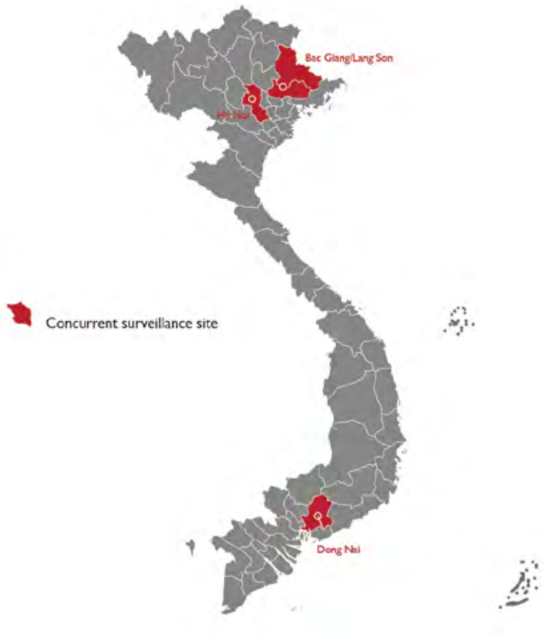
VIRUS GROUP	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS TOTAL	WET SEASON	DRY SEASON
Coronavirus	PREDICT_CoV-35	Unidentified Vesper Bat	Dong Thap	5	5	0
	PREDICT_CoV-47	Unidentified Bat, Wrinkle-Lipped Free-Tailed Bat	Bac Giang	12	12	0
	PREDICT_CoV-82	Wrinkle-Lipped Free-Tailed Bat	Bac Giang	2	2	0
	PREDICT_CoV-99	Unidentified Bat	Bac Giang	1	1	0
	Bat coronavirus 512/2005	Lesser Asian House Bat, Unidentified Vesper Bat	Dong Thap	38	38	0
	Longquan Aa mouse coronavirus	Hoary Bamboo Rat, Norway Rat, Oriental House Rat, Ricefield Rat	Dong Nai, Dong Thap, Thach That	11	11	0
	Murine coronavirus	Black Giant Squirrel, Black Rat, Greater Bandicoot Rat, Hoary Bamboo Rat, Lesser Ricefield Rat, Malayan Porcupine, Norway Rat, Polynesian Rat, Ricefield Rat, Unidentified Rat	Dong Nai, Dong Thap, Thach That	92	90	2
	Rodent coronavirus	Lesser Ricefield Rat, Norway Rat, Polynesian Rat, Ricefield Rat, Unidentified Rat	Dong Thap, Thach That	23	12	11
	Alphacoronavirus 1 (Transmissible gastroenteritis virus)	Domestic Pig	Dong Nai	19	0	19
	Betacoronavirus 1 (Porcine hemagglutinating encephalomyelitis virus)	Domestic Pig	Dong Nai, Dong Thap, Quang Ninh	80	38	42
	Infectious bronchitis virus (IBV)	Domestic Chicken	Dak Nong	2	0	2
Paramyxovirus	Human parainfluenzavirus 3	Human	Dong Nai	1	1	0
	Measles virus	Human	Bien Hoa City Clinic (Dong Nai)	2	1	1
	Mumps virus	Human	Bien Hoa City Clinic (Dong Nai)	1	1	0
	PREDICT_PMV-63	Unidentified Vesper Bat	Dong Thap	1	1	0
	Beilong virus	Norway Rat	Thach That	2	2	0
	Newcastle disease virus	Domestic Chicken	Dak Nong	1	0	1
	Porcine parainfluenzavirus 1	Domestic Pig	Dong Nai, Dong Thap, Quang Ninh, Swine Farms DTH01 & DTH05	7	3	4
Influenza virus	Influenza A	Human, Domestic Pig	Bien Hoa City Clinic (Dong Nai), Dong Nai, Thach That Clinic, Quang Ninh, Swine Farms DTH02 & DTH03 & DTH06	38	26	12
	Influenza B	Human	Dong Nai, Thach That Clinic	2	1	1
Flavivirus	Dengue virus serotype 1	Human	Bien Hoa City Clinic (Dong Nai), Thach That Clinic	6	5	1
	Dengue virus serotype 2	Human	Bien Hoa City Clinic (Dong Nai), Thach That, Thach That Clinic	17	16	1
Total				**363**	**266**	**97**

CONCURRENT SURVEILLANCE & THE WILDLIFE-FARM INTERFACE

The PREDICT project's One Health team worked with national, provincial, and district-level veterinary and medical officers to strengthen multi-sectoral information sharing (a challenge identified in the Joint External Evaluations, a process to assess Viet Nam's capacity to prevent, detect, and rapidly respond to public health risks) and to extend Viet Nam's surveillance system to high-risk areas for zoonotic disease transmission including sites with high rates of wildlife trade and intensive farming of wildlife. PREDICT's concurrent surveillance at high-risk sites contributes to Viet Nam's syndromic surveillance of febrile patients at district and provincial hospitals, screening of people in the community with occupational risk to zoonotic diseases through wildlife trade and wildlife farming, wildlife disease surveillance, and surveillance for pathogens of pandemic potential in livestock in collaboration with FAO and the Department of Animal Health (DAH) of the Ministry of Agricultural and Rural Development (MARD).

WILDLIFE-FARM INTERFACE IN DONG NAI PROVINCE

A high number of wildlife farms are located in Dong Nai Province. The wildlife farms are stocked with wild animals sourced from captive and wild populations. Government agencies, including the Provincial Forest Protection Department and Sub-Department of Animal Health, supported PREDICT's surveillance activities in the wildlife farms and adjacent domestic animal farms; and the Provincial Preventive Medicine Center and Dong Nai Department of Health supported PREDICT's human surveillance in the community and hospitals. The PREDICT team prioritized wildlife farms, nearby livestock, hospital patients, and community members involved in wildlife farming for zoonotic disease surveillance activities due to the close and frequent wildlife-human contact. Targeted sites included districts that have farms that supply three main species (rodents, non-human primates, and civets).



SPECIES COMMON NAME	VIRUS	# SPECIMENS
Human (Hospital)	Dengue virus serotype 1	5
	Dengue virus serotype 2	15
	Influenza A	11
	Measles virus	2
	Mumps virus	1
Black giant squirrel	Murine coronavirus	1
Domestic pig	Alphacoronavirus 1 (Transmissible gastroenteritis virus)	19
	Betacoronavirus 1 (Porcine hemagglutinating encephalomyelitis virus)	35
	Porcine Parainfluenzavirus 1	2
Hoary bamboo rat	Longquan Aa mouse coronavirus	1
	Murine coronavirus	21
Human (Community)	Influenza A	1
	Influenza B	1
	Human Parainfluenzavirus 3	1
Malayan porcupine	Murine coronavirus	6

WILDLIFE MARKET INTERFACE IN HA NOI METROPOLITAN AREA

Ha Noi, with a population of 7.7 million people, is located on an important wildlife trafficking route in Southeast Asia. It is an urban site for the field rat market trade (Animal Value Chain), with numerous people engaged in the capture, sale, and slaughter of the rats. Domestic animals also move through the same markets and restaurants. The PREDICT project conducted surveillance on trafficked animals as well as rodents, which have been listed as a key high-risk taxa for zoonotic pathogens. For these reasons, PREDICT selected Ha Noi as a priority for human, domestic animal, and wildlife surveillance activities. The targeted site was Thach That District, which is the main location of rat consumption and home to many people who are exposed to wildlife on a regular basis during their daily activities.

SPECIES COMMON NAME	VIRUS	# SPECIMENS
Human (Hospital)	Dengue virus serotype 1	1
	Dengue virus serotype 2	1
	Influenza A	7
	Influenza B	1
Human (Community)	Dengue virus serotype 2	1
Norway rat	Beilong virus	2
	Longquan Aa mouse coronavirus	1
	Murine coronavirus	11
	Rodent coronavirus	20
Oriental house rat	Longquan Aa mouse coronavirus	3
Ricefield rat	Rodent coronavirus	2

BAT GUANO HARVEST INTERFACE IN BAC GIANG & DONG THAP PROVINCES

Bac Giang Province has a large cave that provides a roosting habitat for migratory bats. The guano produced by the bats is collected by individuals to use as plant fertilizer. Inside the cave, the bat guano harvesters and cave owners are exposed to overhead droppings and regularly walk through piles of feces with bare feet. The PREDICT team investigated zoonotic disease spillover risk at this natural site and at constructed bat guano farms where the bat guano is collected and harvested by hand (Dong Thap Province). The team used observational research and informal discussions to characterize the guano collection process and identify the roles of individuals within the value chain. Subsequently, we confirmed guano collection as well as adjacent domestic animal holdings as a priority for human, bat, and domestic animal surveillance activities.

SPECIES COMMON NAME	VIRUS	# SPECIMENS
Unidentified bat	PREDICT_CoV-47	8
	PREDICT_CoV-99	1
Wrinkle-lipped free-tailed bat	PREDICT_CoV-47	5
	PREDICT_CoV-82	2

LONGITUDINAL INFLUENZA SURVEILLANCE NETWORK (LISN)

A cohesive understanding of the burden of various priority pathogens, animal/livestock market chains, and the evolution of influenza viruses in Viet Nam requires coordination of animal-human interface surveillance outputs. Therefore, FAO, WHO, and the PREDICT team worked jointly in Viet Nam to link some parts of existing influenza surveillance in livestock, wildlife, and humans with the goal to support the Ministry of Health (MOH) and Ministry of Agriculture and Rural Development (MARD) to enhance detection and characterization for influenza and other viruses with pandemic potential in Viet Nam. This linking of existing surveillance activities is also known as the Longitudinal Influenza Surveillance Network (LISN). LISN also enhances Viet Nam's application of One Health principles to strengthen cross-sectoral collaboration and coordination for both disease outbreak events and routine disease surveillance activities.

After mapping existing surveillance activities and known value chains, the following provinces were selected as sites for implementation:

- Dong Thap Province: South-West Viet Nam: duck grazing activity, movement of spent ducks, rodent trade, and bat guano harvest practices.
- Quang Ninh Province: North-East Viet Nam: movement of chickens, wildlife trade.

SPECIES COMMON NAME	VIRUS	# SPECIMENS
Domestic pig	Betacoronavirus 1 (Porcine hemagglutinating encephalomyelitis virus)	38
	Porcine Parainfluenzavirus 1	1
Greater bandicoot rat	Murine coronavirus	1
House rat	Murine coronavirus	2
Lesser Asian house bat	Bat coronavirus 512/2005	4
Lesser Ricefield rat	Murine coronavirus	7
	Rodent coronavirus	1
	Murine coronavirus	3
	Rodent coronavirus	1
Ricefield rat	Longquan Aa mouse coronavirus	12
	Murine coronavirus	59
	Rodent coronavirus	6
Unidentified *Myotis* bat	PREDICT_CoV-35	5
	PREDICT_PMV-63	1
	Bat coronavirus 512/2005	43
Unidentified *Rattus* rat	Murine coronavirus	2
	Rodent coronavirus	2

OPPORTUNISTIC ACTIVITIES ALONG THE WILDLIFE TRADE CHAIN

Illegal wildlife trade or wildlife trafficking (Animal Value Chain) was an important interface for the PREDICT project. PREDICT established relationships with partners with knowledge of wildlife confiscation events that may involve a variety of wild animal taxa at the destination, source, or transit sites throughout the country. Therefore, as opportunities were available and feasible, PREDICT teams conducted surveillance activities on confiscated wildlife.

SPECIES COMMON NAME	VIRUS	# SPECIMENS
Red junglefowl	Infectious Bronchitis Virus (IBV)	4
	Newcastle Disease Virus	2

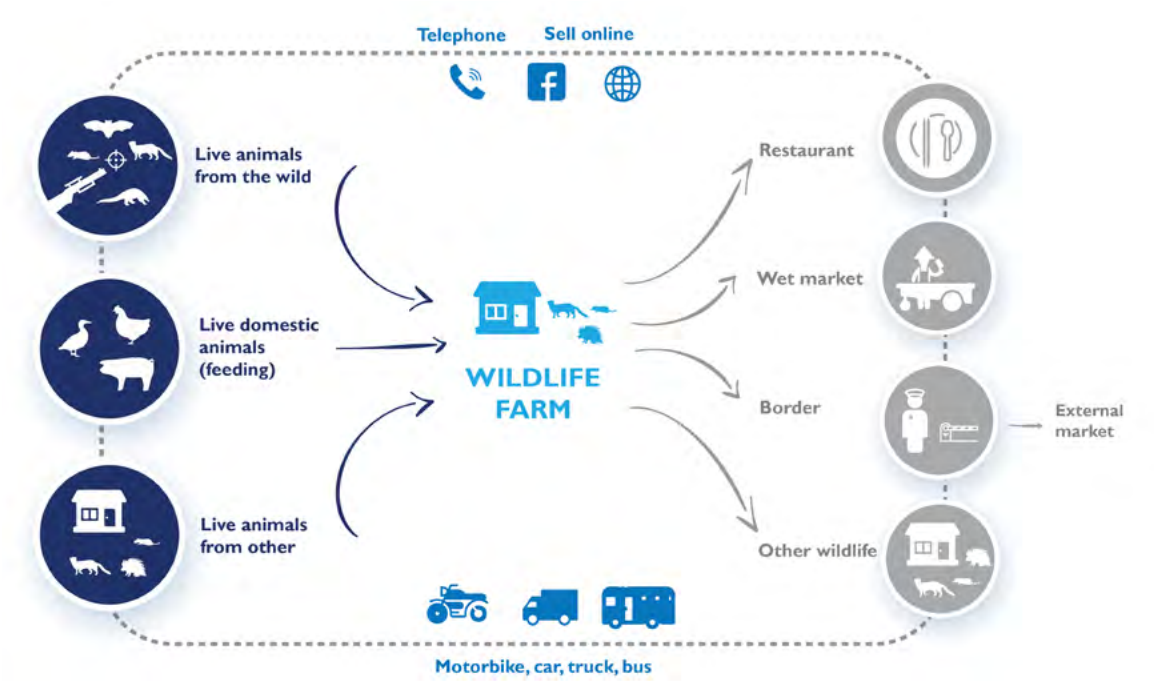
BEHAVIORAL RISK SURVEILLANCE

A primary goal of the PREDICT project was to strengthen global capacity for the detection and discovery of viruses with pandemic potential, specifically those that can move between animals and people (zoonotic viruses). The PREDICT team worked to improve the characterization of associated biological, behavioral, and ecological risks to better understand which geographic locations, 'epidemiological zones', animal-animal and/or animal-human interfaces, and environmental factors are most associated with the evolution, spillover, amplification, and spread of zoonotic viruses with pandemic potential. Ethnographic interviews were used to identify risk factors associated with zoonotic disease transmission risk.

Analysis of PREDICT biological and behavioral surveillance in human populations at key animal-human interfaces potentially associated with the spillover, amplification, and spread of zoonotic viruses is underway. Biological samples were safely collected from humans enrolled in the project for virus testing, and quantitative and qualitative research methods are used to identify risk factors for viral transmission and obtain descriptive accounts of human behaviors and perceptions to support the development of effective public health interventions. Enrollment of humans was part of PREDICT's triangulated surveillance for viruses of pandemic potential in wildlife, domestic animals, and humans at key concurrent surveillance sites.

> "I've only heard about zoonotic diseases from poultry. Such as chicken, pigeon and also other livestock. I brought civets here and then vaccinated them. In my opinion maybe there are zoonotic diseases that pass from civets to humans but my animals are vaccinated so I can have peace."
>
> –Civet farm owner

> "My father told me when I was a child, whenever I was bitten by snake and rat or when I got cut, just suck the blood. He taught me that."
>
> –Rat hunter



Read more about this study here: **www.biorxiv.org/content/10.1101/2020.06.05.098590v3**

In Viet Nam as well as Cambodia and Lao PDR, an important rodent-human interface is the live field rat trade. Based on numbers from the early 2000s the Vietnamese live field rat market was valued at US $2 million and it was estimated that 3,300-3,600 tonnes of live rats were consumed annually (Khiem et al., 2003). This unique zoonotic disease interface involves the capture of wild caught rice field rats and their subsequent trade along a value chain in the Mekong Delta region of these countries. One of the surveillance activities of the PREDICT project focused on this live field rat trade, where we targeted large markets (>20 vendors), trade sites (involved in private sale and processing of live rodents for consumption), and restaurants. Five field rat species were found in the trade (*Rattus argentiventer, R. tanezumi, R. norvegicus, R. exulans,* and *Bandicota indica*). Characterization of which viruses are present and virus group level detection rates along the value chain can help mitigate zoonotic disease risks by informing policy and intervention options.

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ONE HEALTH CASE STUDIES

ACCOUNTING FOR MULTI-SECTOR LOSSES TO INFORM COST-EFFECTIVE DISEASE MANAGEMENT

BANGLADESH

The high costs of recent zoonotic disease epidemics worldwide often extend far beyond the human and animal health sectors. Accounting more fully for these cumulative impacts using a 'societal' perspective can allow for development of cost-effective solutions, particularly for investments to reduce the likelihood and severity of resource-intensive outbreaks. To date, multi-sectoral impact analyses for zoonoses have rarely been conducted at the country level, including in Bangladesh, where financing for disease management is often shaped by single-sector budgeting.

WHAT ONE HEALTH ADDED

Through the PREDICT project, a One Health economics study was implemented for four priority emerging and endemic zoonoses (Anthrax, Influenza A, Nipah, and Rabies), with data collection targeted to districts with a high case burden. By collecting information on direct and indirect impacts at the level of individual affected households and businesses, as well as for public and private sector institutions (11 government and non-governmental institutes, sub-national municipalities, and industry associations), and in the general public, the study captured impacts on a wide range of sectors and stakeholders to understand the type, magnitude, and direction of impacts across different levels of society. Interpreting economic data in the context of risk, industries, and cultural preferences in the country – along with animal and human biomedical and behavioral risk surveillance information collected through PREDICT and other projects in a One Health fashion – can help target risk management interventions to optimize benefits and minimize inadvertent negative consequences.

By employing a consistent methodology across all four diseases but adapting data collection to relevant stakeholders (e.g. case households, farmers/egg sellers/animal value chain, date palm sap collectors, infectious disease hospital, agriculture, environment, and health departments, city corporations, and the tourism industry), the findings may inform coordinated investments for multi-disease management. A partnership with the Institute of Epidemiology, Disease Control, and Research and Department of Livestock Services has been vital to the successful implementation of the study, guiding selection of appropriate metrics and facilitating inclusion of disease cases. It is expected that Bangladesh's One Health Secretariat, which has a rotating leadership structure among three ministries promotes One Health awareness and coordination across sectors, will be a key user of the information generated in the study, potentially linking to the multi-sectoral National Action Plan for Health Security and in the design and financing of zoonotic disease management programs. A 'One Health Economics' approach can also be used for other complex challenges where multiple sectors are relevant for risks or impacts, helping to optimize investments across the Sustainable Development Goals.

PARTNERS:	Institute of Epidemiology, Disease Control and Research, Department of Livestock Services, Bangladesh, EcoHealth Alliance, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; Sustainable Financing

FROM THE FIELD TO THE LABORATORY: CAPACITY BUILDING FOR ONE HEALTH SURVEILLANCE

CAMBODIA

The Royal Cambodian Government's Zoonoses Technical Working Group (ZTWG) notes "Unknown emerging pathogens" as one of their five priority concerns. Although joint teams composed of staff from the livestock and human health sectors have existed to respond to highly pathogenic avian influenza H5N1 outbreaks since the first case in 2005, wildlife was still a neglected component of the One Health triad in Cambodia. With 75% of emerging disease in humans having their origins in animals, and >65% of those coming from wildlife, there was a need for true One Health multisectoral surveillance teams to address this element of the ZTWG's Strategic Plan for Zoonoses Control and building this capacity was a key focus of the PREDICT project.

In the first phase of PREDICT, workshops were held for central and provincial government staff from the human health, livestock health, wildlife and forestry sectors and for veterinary and medical students (the future One Health workforce), to increase awareness of zoonotic diseases, risks for spillover and a preemptive One Health approach to surveillance at their source. In depth, long-term practical training was then provided for students and in-service government staff from medical, epidemiology, veterinary and wildlife departments, from sample collection at interfaces considered higher-risk for zoonotic disease spillover, to sample testing in the laboratory. Training modules included the safe and ethical capture, sampling and re-release of wildlife, including key taxa for zoonotic diseases such as bats and rodents; the appropriate handling and storage of samples and use of cold chain to maintain sample viability for virus discovery when travelling from distant field sites back to the laboratory; the proper use of Personal Protective Equipment (PPE) so important for keeping team members safe; and novel virus group screening methods in the laboratory to identify both known and new viruses. Over 300 people were trained through the PREDICT project in Cambodia. The trained multisectoral field teams from the Ministry of Health and the Ministry of Agriculture, Forestry and Fisheries were the first to conduct true One Health concurrent surveillance of wildlife, domestic animals and humans in Cambodia.

The capacity now exists for multisectoral field teams to conduct One Health surveillance and response together. The government has already adopted the novel virus group screening methods for investigation of outbreaks of unknown origin, including to identify H5N1 in otters at a zoo on the outskirts of the capital city of Phnom Penh. These One Health field and laboratory teams are now positioned to keep Cambodia, and the world, safer from emerging pandemic threats.

PARTNERS:	Ministry of Health. Ministry of Agriculture, Forestry and Fisheries, WCS, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT– 2&3: Real-Time Surveillance; DETECT-5: Workforce Development

DEVELOPING NATIONAL CAPACITY FOR WILDLIFE DISEASE SURVEILLANCE

CAMEROON

Cameroon was one of the first countries in sub-Saharan Africa to adopt a One Health National Strategy in 2012. In 2014, the government created the National Program for the Fight Against Emerging and Reemerging Zoonoses (Zoonoses Program for short) to promote the One Health approach and operationalize the National One Health Strategy and encourage government multisectoral coordination in preventing, detecting, and responding to infectious disease threats. The One Health approach has also been used to identify gaps and weaknesses in the disease surveillance system in Cameroon. Wildlife disease surveillance is one key area identified for capacity enhancement.

WHAT ONE HEALTH ADDED

Multi-sectoral strategic surveillance plans for priority zoonoses (rabies, filoviruses, anthrax and avian influenza) were set up. Cameroon's capabilities for zoonotic disease investigations and multisectoral outbreak response efforts (H5N1, Monkey pox, Anthrax) have been enhanced through field investigations, control activities and planning workshops. However, there was no routine monitoring of wildlife diseases. The PREDICT project helped to convey the value of wildlife disease information in a One Health frame facilitating meetings between ministries of Wildlife and Livestock and promoting incorporation of weekly event-based wildlife disease reports into the national reporting system. To promote feasibility, it was proposed as pilot initiative, providing ideas for target sites and leveraging the valuable existing network of eco-guards that serve on the front lines in forest areas and can implement wildlife mortality monitoring. To support capacity readiness, training was providing to wildlife game rangers from protected areas. The sustainability of the potential program was also reinforced through wildlife disease surveillance training conducted with students in their final year from two veterinary schools, ensuring wider awareness in the veterinary field about wildlife health and disease into the future.

The country's One Health platform is currently in place under the Zoonoses Program, and could be expanded to provide a foundation for taking other major public health concerns like antimicrobial resistance into consideration. In addition to rolling out a wildlife disease program, implementing the One Health approach at the community level and effectively involving the private sector, NGO sector, and security forces are future opportunities for the country to operationalize and benefit from One Health.

PARTNERS:	Ministry of Livestock, Fisheries and Animal Industries, Ministry of Forestry and Wildlife National Program for the Fight Against Emerging and Reemerging Zoonoses, FAO, OHCEA Mosaic, Metabiota, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; Sustainable Financing

ONE HEALTH ACTION RESPONDING TO THE FATAL SWINE ACUTE DIARRHEA SYNDROME (SADS) OUTBREAKS

CHINA

From October 2016, a fatal swine disease outbreak was observed in a pig farm in Guangdong province, China, causing severe and acute diarrhea and vomiting, leading to death in newborn piglets (younger than five days) from rapid weight loss, with a mortality of 90%. By May 2017, the disease had caused the deaths of 24,693 piglets at four farms; both the clinical signs and preliminary lab testing suggested it was a novel disease caused by coronaviruses.

WHAT ONE HEALTH ADDED

PREDICT China joined the outbreak investigations after the first observation. One Health surveillance was conducted to: 1) collect samples from sick pigs to confirm a novel HKU2-related bat coronavirus, swine acute diarrhea syndrome coronavirus (SADS-CoV), as the etiological agent; 2) implement a serosurvey in farm workers; and 3) carry out regular targeted surveillance among bats around the outbreak farms to understand the transmission potential of SADS-CoV from animals to human.

Facing a novel disease caused by an unknown pathogen, the team worked with multi-sectoral partners to quickly identify the pathogen causing disease in pig populations, based on the long-term accumulated coronaviruses database from 10-years of PREDICT work in China; assess the public health risk of the pathogen; and establish surveillance in bats for disease prevention and prediction. This demonstrated an efficient operation through One Health collaboration for emerging infectious diseases response and preparedness.

In addition to the continual surveillance in bats and pigs, retrospective investigation was also conducted to understand the phylogenetic evolution of SADS-CoV. With a rich set of data collected through surveillance, modeling experts helped to understand the amplification dynamics of SADS-CoV, assess its potential for future transmission, and help identify the geographic areas with the greatest potential for bat-pig transmission to mitigate the risk of SADS-CoV and other viral spillover and to prioritize surveillance.

PARTNERS:	Wuhan Institute of Virology, Chinese Academy of Sciences; Beijing Institute of Microbiology and Epidemiology; South China Agricultural University; Key Laboratory of Animal Health Aquaculture and Environmental Control; Wuhan University; Guangdong Laboratory Animals Monitoring Institute; EcoHealth Alliance; PREDICT
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-1: National Laboratory Surveillance; DETECT-2&3: Real-Time Surveillance; DETECT-5: Workforce Development

OPERATIONALIZING ONE HEALTH

CÔTE D'IVOIRE

Significant achievements have been made to advance health security in Côte d'Ivoire, including the President's recent signing of two decrees establishing, allocating, organizing, and operating the public health emergency operations center (Decree No. 2019-292 of 3 April 2019) and the One Health national platform (Decree No. 2019-293 of 03 April 2019). Both show that Côte d'Ivoire has a strong opportunity to build on the willingness of sectors to collaborate and make One Health operational to improve Sustainable Development outcomes. However, One Health in practice remains limited, in part due to the broad scope of human, animal and environmental health and lack of clarity on how and where it can offer optimal benefits.

WHAT ONE HEALTH ADDED

PREDICT organized a workshop at the Institut Pasteur of Côte d'Ivoire (IPCI) on operationalizing One Health in the country. Putting the World Bank One Health Operational Framework to use, the workshop focused on practical, value-added ways to integrate multi-sectoral collaboration, bringing together authorities from Ministries of Health, Veterinary Services, Wildlife, Environment, Security, Rural Development, and Economics and Finance as well as local NGOs and academic partners. An exercise on investing in One Health used three case studies to examine multi-sectoral impacts of African Swine Fever (ASF), lagoon pollution, and impacts from construction of the Soubre dam. For example, communities in northern CIV were heavily impacted by the livestock disease ASF; while not zoonotic, the major losses incurred by smallholder farmers have potentially devastating, immediate, and long-term socioeconomic impacts, including ability to pay for healthcare services, and possible wider pricing implications (e.g. on military food sources). For lagoon pollution, impacts are primarily viewed as environmental or aesthetic, but can be far-reaching, including impacts on food safety and security, water quality, and tourism. Impacts do not necessarily have to be precisely quantified to offer an entry point for discussion and coordinated decision-making on possible solutions to reduce upstream risks as well as downstream impacts.

The workshop identified the need for formalizing support for One Health at the level of the Prime Minister's Office and an optimal mechanism to manage and coordinate the One Health platform and promote additional collaboration in daily functions and planning processes, both in emergencies and peacetime. The need to develop synergistic research and science investigation programs between state agencies and research laboratories (government or universities) was underlined. Workshop participants also discussed the need to invest in human and animal health surveillance system strengthening, and to develop a formal wildlife disease surveillance program. This is a relatively new area of capacity for the country and a plan is needed to ensure that wildlife disease surveillance and risk management be sustained and integrated into human, animal, and environmental health systems to strengthen zoonotic disease surveillance.

PARTNERS:	USAID, IPCI, World Bank, EcoHealth Alliance, Food and Agriculture Organization, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-2&3: Real-Time Surveillance; DETECT-5: Workforce Development; Sustainable Financing

ONE HEALTH PREPAREDNESS FOR EBOLA VIRUS DISEASE OUTBREAKS

DEMOCRATIC REPUBLIC OF THE CONGO

In August 2018, a single human case of Ebola virus disease (EVD) in Beni Territory, North Kivu Province initiated what is now the second largest EVD outbreak in history. One year into the outbreak, EVD had spread to Ituri Province. Despite mass vaccination of people at high-risk for infection (e.g. health care workers and people in contact with EVD patients), the geographic area of the outbreak continued to widen. Ebola virus potentially originates in bats, which carry the virus asymptomatically and shed it intermittently in their saliva, urine and feces, potentially transmitting it to humans and other species.

WHAT ONE HEALTH ADDED

In North Kivu and Ituri provinces, people and wildlife live in close contact. Dense human communities practicing subsistence agriculture live adjacent to forest reserves, and illegal entry into reserves and parks for resource extraction (e.g. charcoal, bushmeat, and water) and occupancy by militia and rebels occurs daily. Some wildlife species, such as bats, thrive in urban and semi-urban environments. In essence, Ebola virus disease is the ultimate One Health tragedy: the circumstances under which the virus spills over from its natural reservoir into people and other wildlife are highly dependent upon how people and animals interact with and utilize the natural environment. Great apes, including chimpanzees and gorillas, are highly susceptible to Ebola, and past human EVD outbreaks have been associated with significant mortality in wild great ape populations. Great apes are infected as humans are: through direct contact with an infected animal, and conceivably through direct contact with infected people.

To date, the role of animals in Ebola virus is not well known by the public. Given the potential for Ebola virus to infect habituated mountain gorillas and cause devastating losses of this endangered species, the PREDICT eastern DRC team took action to build greater awareness of Ebola virus as a One Health pathogen among health professionals and in communities, using these outreach efforts to educate people on the virus and how to minimize risk for infection. PREDICT convened an all-day workshop involving veterinarians, public health workers and laboratory technicians, educating attendees about animals as sources of human disease and the role of veterinarians in helping protect human health. They used the EVD outbreak as impetus for reviewing hygiene practices that help minimize infectious disease transmission. A similar training was held for people working in national parks and forest reserves adjacent or near to the EVD outbreak zone. The team also delivered this material and the *Living Safely With Bats* outreach tool developed by PREDICT to educate more than 300 people on protecting themselves from infectious disease while also living in harmony with wildlife, and this outreach work continues today.

In partnership with Gorilla Doctors, advice was relayed to the Congolese wildlife authority on the threat that EVD poses to gorillas and how best to prevent and respond to the introduction of Ebola virus into gorilla populations. Recognizing that Gorilla Doctors veterinarians are front line health care workers for wildlife, just as those who are working in EVD treatment centers for people, the Provincial government was quick to approve the Gorilla Doctors team in eastern DRC as a priority for vaccination against the virus – a measure of the degree to which EVD as a One Health crisis has been recognized and acted upon by the North Kivu Province government.

PARTNERS:	Gorilla Doctors, UC Davis, PREDICT-2
GHSA:	Zoonotic Disease; DETECT-2&3: Real-Time Surveillance; DETECT-5: Workforce Development; RESPOND-3: Medical Countermeasures and Personnel Deployment

MULTISECTORAL INVESTIGATION OF SUSPECTED LASSA FEVER OUTBREAK

GHANA

In recent years, outbreaks of emerging and re-emerging zoonoses, such as Ebola virus disease, have highlighted the importance of rapid disease detection. Initial spillover of these pathogens from their wildlife reservoir into a single or few isolated cases, presents a small but critical window of opportunity for early intervention to avoid more widespread public health emergencies. Until recently, disease investigations in Ghana were siloed in either the animal or human health sector without communication or coordination across sectors.

WHAT ONE HEALTH ADDED

Since first engaging partners in 2017, the PREDICT project in Ghana has been able to strengthen existing surveillance and diagnostic laboratory capacities for viral zoonoses and serve as a One Health platform in the country. In March 2018, PREDICT responded to a request by the Ghana government to support the investigation of a suspected human Lassa Fever case. PREDICT personnel from the Wildlife Division of the Forestry Commission, Veterinary Services Directorate, and the Noguchi Memorial Institute for Medical Research at the University of Ghana partnered with the Ghana Health Service and the University of Ghana School of Public Health to form a One Health team to assist with the investigation. The team conducted field investigations, safely sampling rodents in accordance with PREDICT's biosafety and rodent sampling guidelines at two sites, Adenta and Ashaiman New Town, where the suspect case was residing prior to his illness. Rodents, including the *Mastomys* sp. reservoir for Lassa fever, were sampled in and around homes and food stores. During the field investigations, the One Health team also administered PREDICT's human questionnaires to local community members to gather information on practices that could be associated with heightened risk for zoonotic disease transmission, and conducted outreach and education to the local community on Lassa Fever and strategies to reduce their risk.

At the time of investigation, testing for the presence of viral pathogens in clinical cases of unknown etiology, especially suspected viral hemorrhagic fever (VHF) cases, were performed in the Virology Department at the Noguchi Memorial Institute for Medical Research, for the Ghana Health Service. In response to the Ebola virus disease outbreak in West Africa in 2014, the Department was equipped for molecular and serological testing for VHFs primarily associated with Ebola, Lassa, Marburg, yellow fever, and dengue. Broader, family-level PREDICT virus screening protocols were implemented to strengthen this capacity by expanding the molecular diagnostic suite of the laboratory to include both conventional and real-time PCR and detect both known and emerging viral threats including paramyxoviruses, coronaviruses, influenza viruses, flaviviruses, and filoviruses. After testing negative for Lassa fever, the patient's samples along with the rodent specimens were screened and also found to be negative for the other high-consequence virus groups using the expanded virus detection platform. This alleviated immediate concerns over a suspected zoonotic disease event while equipping country partners with greater capability for routine surveillance, early detection, and differential diagnosis of known and novel pathogens.

PARTNERS:	Noguchi Memorial Institute for Medical Research; Ghana Health Service; Wildlife Division of the Forestry Commission; Veterinary Services Directorate; University of Ghana School of Public Health; PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-1: National Laboratory Surveillance; DETECT-2 & 3: Real-Time Surveillance; DETECT-5: Workforce Development

ONE HEALTH EDUCATION IN THE FOREST REGIONS OF GUINEA

GUINEA

Large-scale Ebola virus outbreaks are becoming more frequent, suggesting that it may have become endemic in certain regions of Africa, potentially by circulation among animal populations. In addition to identification of these possible animal sources, risk reduction interventions and programs to limit transmission from animals to people are a critical component of increasing the disease detection and response capacity of a country.

PREDICT developed the *Living Safely with Bats* book, a risk communication tool for use in community engagement activities. This book is a collection of prevention measures tailored to local communities to share guidance on how to live safely with bats and avoid exposure to potential zoonotic threats such as the newly found *Bombali ebolavirus* (BOMV). Tools such as the bat book can assist governments to raise awareness about the risks of viral spillover from wildlife and encourage community-based risk reduction activities.

WHAT ONE HEALTH ADDED

In response to the *Bombali ebolavirus* findings in the region, the Government of Guinea specifically requested the PREDICT team to assist with the implementation of targeted community engagement programs. In this context, the team engaged communities in the forest region of Guinea, where surveillance activities took place. They targeted a wide range of at-risk groups including hunters and farmers as well as children. The team held engagement sessions with over 6,800 individuals in 55 communities, of which 3,857 were school children.

All community engagement was done utilizing a One Health approach with risk messaging incorporating aspects of human, animal and environmental health. Key points presented by the team in engagement sessions, particularly with school children, were the importance of reducing risk by limiting contact with bats, but also emphasizing the importance of the role of bats to the health of the environment and ecosystem. The PREDICT team organized activities around these themes such as crayon drawings and plays acting out risk reduction activities. Students were able to further disseminate these key points outside the classroom to friends and family. These efforts will help to raise awareness regarding potential risk-enhancing behaviors among the people most likely to be affected by viral spillover while promoting how to live safely with bats.

PARTNERS:	Viral Hemorrhagic Fever Laboratory of the University of Gamal Abdel Nasser of Conakry in Guinea, University of California, Davis, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease

SUPPORTING BIODIVERSITY CONSERVATION THROUGH SURVEILLANCE & BEHAVIORAL RISK STUDIES

INDONESIA

Indonesia has one of the highest rates of endemic biodiversity. The local demand for wild meat, especially from bats, is particularly high in Sulawesi, the fourth largest island of Indonesia, where high levels of species endemism are observed. During the past decades, the island underwent rapid and unsustainable exploitation of its fauna to supply the wild meat markets of North Sulawesi province. Over-hunting has already extirpated several wild mammal species, including several species of bats from North Sulawesi, and hunting activities consequently expanded to other provinces of Sulawesi to supply the demand from the northern province. Little was known about the role of bat hunting in disease risk, as well as the conservation impact of hunting practices on the island. While there are existing laws requiring permits and quotas for hunting in Indonesia, enforcement is limited, and quotas have not been set for the harvesting of bats.

WHAT ONE HEALTH ADDED

The PREDICT project focused intensively on the bat-human interface in Sulawesi given the high contact rates and potential for pathogen spillover. In coordination with the Ministries of Health, Agriculture, and Environment and Forests, integrated biological and behavioral risk surveillance was conducted with bat hunters, community members, and market sellers. Bats were also sampled and speciated during laboratory testing using genetic barcoding. This study provided the first understanding of the volume of bats in the value chain, indicating that over 1 million bats per year are currently harvested on the island - among them species that contribute important pollination services for maintenance and production of commercially important fruit crops and other flora including tropical forest trees. This first estimate can help conservation managers inform quotas for sustainable consumption; in addition, tracking the value chain and the behavioral practices and perceptions of local communities provides opportunities for potential food and livelihood alternatives, greater enforcement of regulations, as well as critical control points for risk reduction in the value chain if disease risks are identified in the future. This data will be useful to support the Government of Indonesia (MoEF) in the implementation of the regulations related to the wildlife animal trade and use (hunting quota, taxes, and legal permits) in order to improve the sustainability of the wildlife trade. This study also increased awareness of the Directorate General of Livestock and Animal Health Services, under the Ministry of Agriculture, who is responsible for diseases in animals. This study demonstrates how a One Health approach can generate multisectoral collaborations and synergies in data collection and diagnostics that are meaningful to specific sectors as well as for coordinated response, including to fill critical information gaps for biodiversity management.

PARTNERS:	Ministry of Health, Ministry of Agriculture, Ministry of Environment & Forestry, PREDICT-2, Sam Ratulangi University – Manado, Gorontalo State University
GHSA:	PREVENT-2: Zoonotic Disease

INTEGRATING WILDLIFE SURVEILLANCE & PATHOGEN DISCOVERY INTO ROUTINE GOVERNMENT OPERATIONS

INDONESIA

Indonesia has a strong foundation for zoonotic disease surveillance capacity, with extensive experience tackling the threat of avian influenza in wild birds and poultry. For other taxonomic groups and diseases, efforts were previously largely focused on surveillance of rodents, mostly for non-viral diseases such as Leptospirosis. This left the country vulnerable to zoonotic diseases from other hosts.

WHAT ONE HEALTH ADDED

Training on wildlife surveillance and family-level virus screening was provided by PREDICT to both the Ministry of Health (MoH) and Ministry of Agriculture (MoA), helping to expand the Ministry of Health's taxonomic coverage to now include bats as well as expand activities to all provinces of Indonesia for a national surveillance system ("National vector and reservoir surveillance") using sampling protocols developed by PREDICT. Using this capacity, the Ministry of Health discovered several new host bat species for Japanese Encephalitis virus in several provinces, adding clues to the circulation of the disease, which is widespread in mosquitoes across the country. In addition, PREDICT diagnostic protocols were also used by the Ministry of Agriculture for family-level screening, helping to detect a new strain of a porcine virus relevant to livestock production. These examples demonstrate how broader surveillance promoted by a One Health approach – both additional species and broader screening to capture known and unknown viruses – can be mobilized to improve public and animal health authorities' understanding of the epidemiology of pathogen transmission. The training, which has been upscaled for wider capacity through a 'train-the-trainer' approach, has also helped researchers in the country reduce reliance on lethal sampling, allowing for safe and humane surveillance that is not detrimental to wildlife populations.

PARTNERS:	Ministry of Health, Ministry of Agriculture, Ministry of Environment & Forestry, FAO, PREDICT-2, LIPI
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-1: National Laboratory System; DETECT-5: Workforce Development

ESTABLISHING A ONE HEALTH PLATFORM FOR MULTI-SECTORAL INFORMATION SHARING & INVESTIGATION

JORDAN

A bright side of PREDICT Jordan is the establishment of focal point partners from the Ministry of Agriculture (MOA), Ministry of Health (MOH), Ministry of Environment (MOE), World Health Organization (WHO), Food and Agriculture Organization (FAO), World Organisation for Animal Health (OIE) and Royal Scientific Society of Jordan (RRS). This committee now serves as a platform for future implementation of the One Health approach in the country. It meets regularly to update all partners about the project's progress and findings for further assessment and possible national policymaking. This is the first national committee for One Health in Jordan and the entire region. The progress and future of the One Health platform initiated by PREDICT Jordan was discussed with all partners, with its value recognized in the decision to hand over the One Health committee to the Ministry of Health post-project to continue the efforts and promote the committee's long-term sustainment.

WHAT ONE HEALTH ADDED

Enhanced surveillance and in-country detection capacity development through PREDICT and its partners have supported the country in becoming a leader in investigating emerging and re-emerging viral infections of both human and animal origins. This capacity has reduced the time necessary to obtain valuable diagnostic results, and at a significantly lower cost since scientists and researchers from Jordan used to send such samples to international, specialized laboratories for cloning and sequencing. For example, during May 2016, the PREDICT team, FAO, Kansas State University, and the National Institute of Health (NIH) Rocky Mountain Laboratories conducted a nationwide study where blood samples from camels in Ramtha and Azrak were collected and tested for MERS-CoV. Out of 120 samples, 36 were positive. This was the first-ever reporting of this disease to OIE in camels in Jordan. To date, only a few countries have reported virus-positive MERS-CoV test results to the OIE, so this is a significant and important step in improving both MERS-CoV detection and reporting in the Middle East. Furthermore, the MOH sampled humans from the two sites to screen for MERS-CoV. The results were published in Vector Borne Zoonotic Disease Journal (2017 Feb; 17(2):155-159) under the title "High Prevalence of Middle East Respiratory Coronavirus in Young Dromedary Camels in Jordan".

In addition to mobilizing multi-sectoral teams to investigate high-consequence zoonotic pathogens of concern for public health, the country's expanded virus detection expertise has been leveraged for the investigation of threats to environmental health and food security. For example, in November of 2018 millions of carp fish died along the length of the Euphrates and Shatt al-Arab Rivers in Iraq. While poisoning was initially suspected as the cause of the disastrous die-off, which affected a key food source in Iraq, the JUST laboratory was the first to detect Koi herpes virus for the first time in Iraq as the causative agent behind the death of the fish in this outbreak. Jordan's agility in supporting investigation across species reinforces the value of One Health coordination and capacity for strong and prepared human, animal, and environmental public health systems in the region.

PARTNERS:	JUST; RSS; Ministries of Agriculture, Environment, and Health; FAO; OIE; WHO; NIH; Kansas State; EcoHealth Alliance; PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; PREVENT-3: Biosafety and Biosecurity; DETECT-1: National Laboratory Surveillance; DETECT-2&3: Real-Time Surveillance; DETECT-5: Workforce Development

TRAINING THE NEXT GENERATION OF ONE HEALTH PROFESIONALS

KENYA

Strengthening capacity for One Health research in Kenya entails spurring the professional development of tomorrow's One Health heroes. To safeguard public health and effectively respond to the growing infectious disease challenges of the modern world, Kenya's future One Health workforce must be resilient, adaptable, and able to work across multiple fields to achieve shared public health goals and positive health outcomes for all.

WHAT ONE HEALTH ADDED

To train the next generation of Kenya's One Health professionals, PREDICT worked with the One Health Central and East Africa (OHCEA) network and Nairobi-based universities to develop and host a One Health field course for graduate-level public health students. Situated at the PREDICT virus sampling and behavioral risk study sites in Laikipia County, the workshop aimed to bridge students of diverse disciplines, enabling them to share perspectives, connect personally with the communities whose health they were tasked with protecting, and become familiar with field applications of public health. The course was an important learning opportunity for students, who were invited into rural communities by health liaisons to identify One Health challenges – including risk factors arising from living conditions, behavioral practices, and occupation – and produce practical solutions to mitigate the risk of zoonotic diseases. By visiting these communities, students were inspired by their experiences to begin generating their own research questions and interests. The course also offered an opportunity to observe and participate in community outreach, providing students with educational tools and public messaging strategies that can be adapted to One Health objectives. They were able to impart on community members the importance of biosafety and biosecurity, and discussed the role of their behavioral practices in their risk of zoonotic diseases. In addition to disclosing PREDICT Kenya project findings, OHCEA provided vaccinations for companion dogs and cats to safeguard communities against rabies.

This education initiative was instrumental in developing observational, field, and communication skills and encouraging open collaboration among participants. Seven faculty and 25 students attended the workshop. Their backgrounds ranged widely, including civil engineering, rangeland management, journalism, environmental policy, social sciences, veterinary and human medicine, biochemistry, and virology. Each provided unique perspectives on health priorities and contributions to solutions-building, leading to multi-pronged approaches to collectively manage community health. Our collaborators also secured the buy-in of local government parties, including Laikipia County's Executive Committee Member (CECM) in charge of health making a guest appearance to offer policy perspectives on community health. With this course a resounding success, we hope inter-agency partnerships with universities will allow these field education opportunities to continue. By training students in the skills necessary to protect public health, we are ensuring a sustainable and lasting impact on Kenya's One Health capacity.

PARTNERS:	Institute of Primate Research; Ministry of Agriculture (Directorate of Veterinary Services); Zoonotic Disease Unit (ZDU); Ministry of Health (County Government Public Health); One Health Central and East Africa (OHCEA); University of Nairobi; Kenyatta University; Moi University; Mpala Research Centre; UC Davis; PREDICT; Global Health Program, Smithsonian Conservation Biology Institute; Mawazo Institute
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-5: Workforce Development

IT TAKES A VILLAGE: EMPOWERING KENYA'S CHILDREN THROUGH ONE HEALTH EDUCATION

KENYA

Upon completion of PREDICT Kenya's field sampling activities, attention was directed towards using our results to advise communities on how to better protect themselves from zoonotic disease outbreaks. In early 2019, project staff hosted community engagement, outreach, and education events for those communities in Laikipia County who participated in our surveillance and behavioral risk investigation: Lekiji, Mpala, Ol Jogi, and Ilmotiok. These predominantly agrarian or pastoralist societies were situated in rural areas, with typically limited access to healthcare services. While community leaders and members certainly benefited from messaging on mitigating zoonotic disease risk, we hoped to make an even stronger impact on the children in the communities.

WHAT ONE HEALTH ADDED

As part of our community engagement activities, we visited three primary schools in the Laikipia region, reaching over 500 students with our One Health messaging. We used a variety of visual education tools to discuss zoonotic diseases, their sources, and ways to protect individuals and communities from infection. These included screening a documentary film, displaying a mobile "Outbreak" exhibit customized to feature infectious disease hazards specific to the east Africa region, and presenting educators with copies of the PREDICT book *Living Safely with Bats* as an additional tool for disseminating information to their pupils. These were followed by discussion sessions where students were invited to ask questions and offer their thoughts on the content, leading to discourse on ways to stay safe, characteristics of specific pathogens such as Ebola virus, and the diversity of bats and the viruses they carry. Students were receptive to the material and keen to learn. Our activities also contributed to another objective: to develop their scientific inquisitiveness and encourage students to pursue further education opportunities, when possible.

Ultimately, we aim to empower communities to make the most informed decisions about their own health, and inclusion of One Health in primary school curricula is a critical step towards that goal. Public health is the responsibility of all members of a community, and by targeting our messaging towards primary school students, we aim to encourage the adoption of risk-mitigating strategies from a young age, thus propagating knowledge of effective disease prevention.

PARTNERS:	UC Davis; Global Health Program, Smithsonian Conservation Biology Institute; Smithsonian's National Museum of Natural History; Mpala Research Centre; One Health Central and East Africa (OHCEA); Institute of Primate Research; PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-5: Workforce Development

PROACTIVE PATHOGEN DETECTION & RISK COMMUNICATION TO PROTECT COMMUNITIES LIVING CLOSELY WITH ANIMALS

LIBERIA

Liberia was one of three countries heavily impacted by the Ebola epidemic in West Africa from 2014-2016. The initial spillover event was known to have occurred in Guinea, yet the specific animal source(s) of the epidemic remained speculative. Through wildlife sampling under PREDICT's filovirus-intensive surveillance effort in Liberia, a Greater Long-fingered bat (*Miniopterus inflatus*) sampled in the northern territory of Liberia tested positive for *Zaire ebolavirus*. This is the first evidence of Ebola virus detected in a bat in West Africa. Proactive measures were taken by the Government of Liberia in response to the finding. This included immediate and diligent collaboration for review, interpretation, and reporting of the finding. The public release of information was done by the Ministry of Health and National Institute for Public Health in Liberia and was paired with a risk communication strategy to reach the communities in the surrounding region to in an effort to reduce possible exposure from bats and minimize unintended consequences.

WHAT ONE HEALTH ADDED

PREDICT's support of the first wildlife veterinarian in Liberia led to critical workforce development where veterinary capacity is extremely limited. Ten field staff have been trained on PREDICT animal handling, sampling, and biosafety protocols and these trainings have been deployed for use in extensive bat and rodent sampling. In addition to continuing to shed light on the role of bats as the reservoir for a high-consequence pathogens, the capacity of the trained wildlife disease investigation team was leveraged to provide research and management support for other zoonotic diseases, including Rabies. Along with wildlife surveillance, the social science component of PREDICT examined the behaviors, practices, and ecological and biological factors driving disease emergence, transmission, and spread through investigating human and animal interactions, knowledge of disease risk, and the use of personal protective measures. The team interviewed respondents in eight counties, gathering information on how they interacted with animals before and after the Ebola crisis. Using a multidisciplinary approach, high-risk sites were identified to enroll people with exposure to wildlife or at land conversion sites with the potential for exposure to emerging pathogens to get at the 'how' and the 'why' of disease risk. The team assessed community perceptions of animal exposure and disease risk and evaluated widely held assumptions of community practices (e.g. high-risks from bushmeat hunting). This integrated biological and behavioral approach can provide enhanced understanding of disease spillover and transmission dynamics, essential to the design and evaluation of mitigating interventions, and inform policy options by identifying barriers to change and acceptable alternatives.

Liberia has embraced the One Health approach in light of the country's endemic and emerging zoonotic disease priorities, forming a One Health Secretariat with representation from human and animal health, forestry, and environment agencies. This provides a strong platform to improve understanding of baseline risk, needs assessment for additional priority surveillance, and interventions to target high-risk activities. For example, Ebola is now included in the National Animal Health Surveillance Strategy developed in early 2019. The Ebola finding is also potentially relevant for the health and conservation of Liberia's wildlife as other species are susceptible to Ebola virus.

PARTNERS:	Society for the Conservation of Nature in Liberia, Ministry of Health, National Public Health Institute of Liberia, Forestry Development Authority, Liberia Chimpanzee Rescue and Protection, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT– 2&3: Real-Time Surveillance; DETECT-5: Workforce

UNDERSTANDING PATHOGEN CIRCULATION & EXPOSURE RISKS IN AN INDIGENOUS POPULATION

MALAYSIA

The Orang Asli are the indigenous people of Peninsular Malaysia and account for 0.61% of the total population. Orang Asli communities are often remotely located, and in heavily forested areas. The Orang Asli people practice traditional, and in many cases, subsistence hunting of wild animals and raise domestic animals such as chickens and hunting dogs around their villages. Their health and nutritional status tends to be far worse than the rest of Malaysia. Many of these communities have limited access to medical services which means disease spillover events could be going unrecorded. Because of the high degree of contact with wild and domestic animals through hunting and rearing practices the Orang Asli are vulnerable to infection with zoonotic pathogens. Encroachment on their ancestral lands for agricultural expansion and mining leads to increased contact and conflict with wildlife, contamination of their water supplies and disrupts their traditional way of life, increasing their vulnerability.

WHAT ONE HEALTH ADDED

Surveillance of Orang Asli communities represents a critically important opportunity to detect spillover of zoonotic viruses. They are not entirely isolated from the rest of Malaysia, traveling for education, work, health care, or to visit other communities. As these communities become increasingly connected through agricultural expansion, urban spread, and an improved road network they become increasingly important as a sentinel population. To improve understanding of pathogen circulation and exposure risks, PREDICT Malaysia conducted concurrent animal-human sampling in Orang Asli villages. Behavioral surveys were conducted to identify perceptions and practices around wildlife, domestic animals, hunting, and livelihood. Working with our partners from the Ministry of Health (MOH) District Health Teams (from Gua Musang, Kuala Lipis and Kuala Kangsar), the Department of Wildlife and National Parks (DWNP) and the Department of Veterinary Services (DVS), we sampled 1,391 people, 736 wild animals (bats and rodents) and 695 domestic animals and pets, including dogs, chickens, goats, wild boars, monkeys and a masked palm civet.

Despite the remoteness of many of the sites and the logistical challenges they posed, the need for multiple visits to each village to meet community leaders to explain the study and how it would benefit their communities, the team managed in just two dry seasons to do what many thought was not possible. This huge sampling effort helped strengthen the One Health Partnership between MOH, DWNP and DVS and provided an opportunity to talk to over 1,000 Orang Asli villagers at community meetings about zoonoses, the One Health concept, and the risks posed by contact with wildlife and how to minimize these risks, such as not touching dead wildlife.

Gaining the trust of these communities generated an entry point for continued engagement. Each sampling trip started with a community meeting and meal to share information about zoonoses and also provided a chance for these communities to get to know the team. Participants were given a health assessment, and provided with treatment by the District Health Teams, providing a pathway for improved access to health care. Returning to each community to share results with participants and a summary of project findings strengthened this relationship and provided another opportunity to share health information such as the PREDICT guide *Living Safely with Bats*. This work demonstrates how One Health novel pathogen surveillance and outreach can be combined with wider public health service delivery for known disease threats to tackle overall health needs.

PARTNERS: EcoHealth Alliance, Ministry of Health, Gua Musang District Health Team, Kuala Lipis District Health Team, Kuala Kangsar District Health Team, Department of Wildlife and National Parks, Department of Veterinary Services, PREDICT-2

PHOTO CREDIT: WCS Mongolia

INTEGRATING WILDLIFE CONSIDERATIONS INTO PPR CONTROL

MONGOLIA

In early January 2017, environmental rangers were encountering an unprecedented number of sick and dead saiga antelope (*Saiga tatarica mongolica*) – a critically endangered species - as they drove their survey transects. It was quickly determined that the disease killing the saiga was peste des petits ruminants (PPR), a pathogen that had first been confirmed in Mongolian sheep and goats in 2016. The outbreak of PPR in Mongolian saiga represents the first time the disease was recorded in a free-ranging antelope species and took both the wildlife conservation and livestock health experts by surprise. The confirmation of PPR virus in Mongolian saiga, and other wild ungulate species in January 2017, was an indication that the large-scale vaccination of livestock for PPR completed in October 2016 had not been effective in stopping on-going transmission in Mongolia, putting non-vaccinated sheep and goats and susceptible wildlife species at risk of infection.

WHAT ONE HEALTH ADDED

Mongolia took a multidisciplinary and multi-sectoral approach to addressing PPR emergence at the wildlife-livestock interface in Mongolia. A national emergency management committee activated an inter-agency response to the PPR die-off in wildlife. The Ministry of Environment was nominated by the Emergency Management Authority to take a leadership role coordinating support from the agricultural ministry, animal health laboratory network (national and international), wildlife experts (national and international), and wildlife-focused NGOs. Mongolia has a history of working across sectors and applying a "One Health" approach to disease at the livestock/wildlife interface, including coordinating avian influenza surveillance in wild birds with veterinarians and environmental agency officers/rangers. This foundation was used to adapt and respond to PPR in wildlife for initial investigations and going forward for monitoring and prevention.

The PREDICT lead in-country is playing a leadership role in encouraging, supporting, and advocating for the multi-sectoral response to the die-off in saiga antelope and other wildlife and linking expertise and resources in the agricultural sector to address PPR in Mongolia. Sharing of laboratory data and joint analysis of data from wildlife and livestock have been initiated and are on-going. Through the PPR response and monitoring, environment sector rangers and local veterinarians have both been mobilized. This is an evolving situation with opportunities for integration not only in the response to PPR in Mongolia but also in the longer-term management and prevention of PPR at the livestock/wildlife interface. Looking at PPR through an integrated approach (considering wild species) will likely be essential to the success of eradication (a livestock sector goal linked to livelihoods, and local and regional economies). The PPR Global Eradication Programme is in the process of formally incorporating wildlife into its guidelines for implementing national and regional prevention and control strategies and advocating for a more "ecological" approach to disease eradication.

PARTNERS: Wildlife Conservation Society; Ministry of Nature, Environment, and Tourism; Ministry of Food, Agriculture, and Light Industry; State Central Veterinary Laboratory; National Emergency Management Agency; WWF Mongolia Program Office; Livestock herders, local veterinarians and rangers; PREDICT-2; FAO; OIE; Morris Animal Foundations; SNAPP

GIVING ENVIRONMENTAL HEALTH AN EQUAL VOICE FOR A STRONGER ONE HEALTH IMPLEMENTATION

MYANMAR

With recent political and economic changes, the human-wild animal interface in Myanmar has signifcantly increased, thus increasing the risk for zoonotic disease transmission and outbreaks. One Health is being viewed as an area in need of further development and strengthening through multisectoral collaboration of responsible Ministries. A key challenge remains in recognizing the need for comprehensive interventions for early notification and prevention of zoonoses. While traditional practices and lack of awareness in communities put the population at high-risk for zoonotic disease transmission, attention has mainly centered around livestock-related disease due to the visible loss of business and livelihoods, rather than the general population and wildlife.

WHAT ONE HEALTH ADDED

To address the apparent gap at the wildlife-human and wildlife-livestock interface, One Health partners (health and livestock ministries) in Myanmar welcomed the PREDICT project. Representatives from the Ministry of Natural Resources and Environmental Conservation (MONREC) were invited to participate in national One Health implementation meetings marking the first time MONREC began directly participating in the protection of human health thus adding a significant voice towards addressing One Health concerns in Myanmar. The PREDICT team organized a series of consultations with three relevant ministries to advocate for a more inclusive One Health approach and explore collaboration on technical and programmatic areas of the project, thereby informing practical and sustainable pathways for the One Health concept, which is still very new in this country. The success of gaining acceptance and interest of policy makers in awareness-raising and capacity building related to environmental and wildlife outbreaks has been remarkable. The participation of PREDICT in the development of the One Health National Strategic Plan (2017-2022) effectively bridged the gap between advocacy and capacity strengthening related to wildlife and environmental health – a vital but often missing sector of the One Health approach. The environment ministry acknowledged that their active participation as a main counterpart in One Health implementation will be crucial and value-added with the application of evidence-based information for assessment and management of risks.

Through the capacity enhanced under PREDICT, trained and well-informed health staff and community health workers can identify wildlife-humaninterface risk areas and potential at-risk populations to target surveillance. Dissemination of project findings to key partners and communities further promoted awareness of zoonotic disease transmission risk within their daily lives. This work demonstrates that instead of a one-size-fits-all approach, modifying disease prevention and mitigation to specifically suit Myanmar's conditions and context can reduce risks upstream and promote early warning for disease threats. In this way, potential partners can better see the value of their particular role in One Health implementation. Early detection of threats at their source can prevent disease spillover events and result in fewer outbreaks. Such a progression contributes to a country's ability to control disease spread and reduce risk of epidemics in the future.

PARTNERS:	Department of Medical Research, Ministry of Health and Sports, Livestock, Breeding, and Veterinary Department (LBVD), Ministry of Agriculture, Livestock, and Irrigation, Forestry Department, Ministry of Natural Resrouces and Environmental Conservation, Central Epidemiology Unit, Department of Health, MOHS for One Health National Strategic Plan (2017-22), University of Veterinary Science, Yezin, University of Medicine 1, Yangon, Zoology Department, Yangon University, WHO, FAO, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-2&3: Real-Time Surveillance

BUILDING DISEASE DETECTION & CHARACTERIZATION CAPACITY

NEPAL

One of the major objectives of PREDICT Nepal has been to develop capacity for disease detection and outbreak response. This has been helpful in building the necessary groundwork for establishing disease surveillance systems to address potential zoonotic disease outbreaks, including through the detection and characterization of viral pathogens.

WHAT ONE HEALTH ADDED

In March 2017, there was an H5N8 outbreak starting from a layer farm in the southeastern part of the country. The virus hit layers in two different sheds, one housing 40-week-old birds and the other containing 20-week-old poultry. Of 6,200 susceptible birds, the virus killed 3,650, and authorities culled the remaining ones to contain the spread of the disease. Rapid response teams also destroyed eggs, feed, egg trays, and some local hens. Upon a formal request from the government-owned Central Veterinary Laboratory, the PREDICT Nepal team assisted their laboratory with molecular detection and characterization of the virus which helped them to rapidly contain the outbreak. Previously the government lab was relying on detection analysis being done by labs outside of the country, which often took several weeks; the in-country partnership on resource sharing and technical collaboration has resulted in rapid detection and characterization of pathogens within a few days. This has greatly enhanced the turnaround time in outbreak mitigation and containment.

PARTNERS:	Food and Agriculture Organization, the Department of Livestock Services, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT– 2&3: Real-Time Surveillance; DETECT-5: Workforce Development; RESPOND-2: Linking Public Health with Multisectoral Response

ESTABLISHING & PROMOTING ONE HEALTH AT A NATIONAL LEVEL

NEPAL

In 2014 PREDICT supported Nepal's first workshop entitled "Feasibility of One Health (OH) initiative in Nepal for surveillance of emerging and re-emerging zoonotic diseases of public health importance". Previously, there was significant interest in One Health approaches but hurdles remained in national and local policy and operationalization of this approach in on-the-ground surveillance and response activities.

The dialogue resulting from this workshop catalyzed the "One Health Alliance for Nepal" which subsequently gained momentum on bridging gaps between members from the Ministry of Health, the Ministry of Livestock and Agricultural Development, and the Ministry of Forest and Soil Conservation, as well as numerous interested private organizations. This Alliance has been leveraged for progress on multiple priorities, including capacity strengthening, risk characterization, and outbreak response. In addition, Nepal is one of the founding members of the One Health Network in South Asia. PREDICT provided an opportunity for Network members to come together to share experiences and best practices from the region, helping to promote collaboration and widen the country's reach for upscaling and sustainability at the regional level.

WHAT ONE HEALTH ADDED

Nepal is a biodiversity-rich country boasting 10 national parks, four wildlife reserves and six conservation areas, occupying 23.3% of the country's total land area. Many national and international agencies and organizations have long supported wildlife conservation and management in the country, contributing to extensive research on Nepal's wildlife and ecology. Wildlife health and wildlife diseases, however, have been given much less attention, despite changing climate and human practices in Nepal that have greatly increased disease threats to wildlife as well as the transmission of diseases among wildlife, domestic animals, and humans.

Data on wildlife policy and gaps in wildlife health were collected from key informants in the government. Following data analysis, PREDICT assisted in organizing a three-day strategic planning workshop organized to discuss current wildlife health capacity issues and needs, as well as strategies to address them. Noting that a policy was lacking for disease surveillance in wildlife, stakeholders agreed to work closely in formulating a national plan from a One Health perspective and drafted the National Strategies for Wildlife Disease Control. The government of Nepal is working on integrating this into their national wildlife and One Health strategies to address current gaps in priority disease surveillance and reporting.

PARTNERS:	Food and Agriculture Organization, the Department of Livestock Services, the Epidemiology and Disease Control Division, the Agriculture and Forestry University, the National Agricultural Research Council, the Himalayan College of Agricultural Sciences, PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-5: Workforce Development

RENFORCEMENT DE LA SURVEILLANCE ET DES LABORATOIRES COMME APPUI MULTISECTORIEL

RÉPUBLIQUE DU CONGO

Les capacités de santé publique en République du Congo (Congo) sont confrontées à des défis critiques, notamment la faible densité du personnel de santé et des infrastructures limitées des laboratoires. Les interfaces de contact entre l'homme et la faune sauvage sont très présentes dans de nombreuses régions du pays, et le pays a connu des épidémies de maladies d'origine zoonotiques, y compris une épidémie de variole du singe (Monkeypox) dans le nord du pays en 2017. Toutefois, le pays a récemment fait des progrès importants en matière de prévention et de préparation aux zoonoses, en utilisant une approche " Une seule santé " pour promouvoir des améliorations des capacités en santé publique qui profitent à de multiples secteurs.

CE QUE UNE SEULE SANTÉ (ONE HEALTH) A AJOUTÉ

Le projet PREDICT a été un pionnier en matière de plaidoyer et de promotion de l'initiative One Health in Congo, en créant avec succès un Consortium multisectoriel One Health impliquant les Ministères de la défense, de la santé, de l'agriculture, des forêts et de la faune, de l'environnement, de la recherche scientifique, des finances, le ministère des affaires étrangères, l'OMS et la FAO. Après la mise en place du Point Focal du Règlement Sanitaire International (PF RSI) qui fournit une plateforme multisectorielle pour la coordination de la préparation et la riposte des épidémies, ces discussions du Consortium ont servi de modèle pour la tenue des réunions hebdomadaires du PF RSI. Ce comité est présidé par le directeur général de l'épidémiologie et du contrôle des maladies et de l'intervention.

Les activités du Laboratoire national de santé publique (LNSP) ont également intégré les approches d'une seule santé. Auparavant, le laboratoire diagnostiquait certains pathogènes. PREDICT a mis en place le premier laboratoire dans le pays dédié a la détection de nouveaux virus et a formé le personnel afin qu'il puisse répondre aux besoins des Ministères de la Santé, de l'Agriculture et des Forêts et soutenir les efforts du Ministère de la Défense. Ce renforcement a permis d'accroître les capacités du pays en matière de surveillance, de diagnostic et de biosécurité, y compris la formation et la mise en œuvre de solutions d'infrastructure pour promouvoir la fiabilité et la sécurité du laboratoire (p. ex. la production d'énergie de secours pour éviter l'instabilité de l'énergie à l'unité de biologie moléculaire, qui était auparavant un défi majeur pour des activités de laboratoire continues, la formation à l'utilisation de GPS et au prélèvement d'animaux sur le terrain, et l'utilisation d'une chambre froide fermée pour la conservation des échantillons en sécurité). Les améliorations apportées à l'équipement de laboratoire ont été mises à profit lors de la détection et de l'intervention lors de l'épidémie de chikungunya en 2019, démontrant ainsi les avantages d'une capacité qui favorise la préparation à plusieurs menaces sanitaires. Il existe à ce jour un intérêt grandissant à la formation du personnel et à l'analyse d'échantillons provenant du ministère de l'Agriculture afin de favoriser la collaboration entre secteurs. Le laboratoire teste également des carcasses d'animaux sauvages, qui est reconnue comme une activité de santé publique importante dans le pays, étant donné les découvertes antérieures au Nord de la Congo qui ont montré que le décès de grands singes peut précéder les cas humains dus au virus Ebola, fournissant un système " d'alerte précoce " pour informer les stratégies visant à réduire la menace à la santé publique.

PARTENAIRES:	Laboratoire National de Santé Publique, Wildlife Conservation Society EcoHealth Alliance, PREDICT-1 et PREDICT-2
GHSA:	PREVENT-2 : Maladies zoonotiques ; DETECT-2 et 3 : Surveillance en temps réel ; DETECT- : Renforcement des capacités ; RESPOND-2 : Relier la santé publique à la réponse multisectorielle

VIRUS X HUNTERS: OPERATIONALIZING ONE HEALTH IN EMERGENCY RESPONSE & SURVEILLANCE

SIERRA LEONE

In 2014 Ebola virus (*Zaire ebolavirus*) caused a deadly outbreak that took the lives of over 4,000 people in Sierra Leone. While the natural reservoir host of the virus is still unknown, the evidence base suggests that certain species of bats are the likely source, with suspected circulation in animal populations in West Africa. Rapid control of any future spillover event is dependent on a number of factors, including the strength of infectious disease surveillance and response capacity in the region, and understanding which animals play a role in the maintenance and transmission of the virus.

WHAT ONE HEALTH ADDED

In Sierra Leone, the Ebola Host Project (EHP), which is part of the larger PREDICT project, was implemented to identify the animals that may act as reservoirs or transmission hosts for Ebola and other filoviruses, to further understand the spread of the virus and recommend prevention measures as well as strengthen in-country capacity to prevent, detect and respond to emerging threats. This focused effort resulted in discovery of a new ebolavirus species, *Bombali ebolavirus*, in a bat host. To date, this virus has not been detected in a human or sick animal and the potential to infect people is not known. Just months after that discovery, the project detected Marburg virus, a known and highly-lethal filovirus, for the first time in the country in collaboration with the US Centers for Disease Control and Prevention (CDC) and Njala University. This virus was found in "Egyptian" fruit bats (*Rousettus aegyptiacus*), which are known to feed mainly on fruit and nectar and can shed Marburg virus in their saliva, urine, and feces. These discoveries indicate the value of pathogen detection work – for known and unknown pathogens from high-consequence virus groups – outside of disease emergencies to understand baseline disease risks in communities and promote public health preparedness.

The findings build on close collaborations established with the Ministry of Health and Sanitation, Ministry of Agriculture, and Ministry of Forestry and provide a foundation for tracking filovirus threats and developing risk reduction strategies. Over 160 government officials have been trained on One Health skills in six districts throughout the country through the PREDICT project. With an increased trust between the Ministry partners through open collaboration and communication, PREDICT was embedded in the Ministry of Health's Public Health National Emergency Operations Centre (PHNEOC) within the Directorate of Health Security and Emergencies, to serve as technical experts in One Health and zoonoses surveillance. Updates on filovirus monitoring are invited during weekly Emergency Preparedness Rapid Response Group (EPRRG) meetings, recognizing the utility of this information. Partners and stakeholders meet together to communicate and monitor epidemiological human and animal events throughout the country and make suggestions to senior management, with a goal of staying proactive before any zoonotic disease spillover into humans.

PARTNERS: University of Makeni, Ministries of Health and Sanitation, Agriculture and Forestry, U.S. CDC, Njala University, University of California, Davis, PREDICT-2

GHSA: PREVENT-2: Zoonotic Disease; DETECT– 2&3: Real-Time Surveillance; DETECT-5: Workforce Development; RESPOND-1: Emergency Operations Centers; RESPOND-2: Linking Public Health with Multisectoral Response

WORKFORCE TRAINING TO ADDRESS ANIMAL SURVEILLANCE CAPACITY NEEDS

TANZANIA

As the first country to host a Joint External Evaluation (JEE) of the International Health Regulations Core Capacities and develop a multi-sectoral National Action Plan for Health Security, Tanzania is a leader in its commitment to health security. Surveillance in the human health sector has significantly improved since the JEE in 2016, with real-time surveillance and reporting enhancements for priority diseases. Gaps still remain in linking human cases to animal or environmental exposures at the subnational and community level, in part because of a front line workforce shortage: only 6% of villages have animal health staff, contributing to limited communication and sharing of information between institutions.

WHAT ONE HEALTH ADDED

In 2019, PREDICT hosted a One Health specialist short-course for 26 national and subnational managers in Tanzania. The workshop integrated laboratory staff (human and animal), regional and district-level veterinary staff, medical doctors, nurses, health port officers, and wildlife researchers to strengthen One Health multi-sectoral communication and to enhance technical skills associated with disease surveillance and response.

Due to the specificity of skills covered, participants were split into groups for targeted field and human and animal laboratory training, but came together to work on community engagement for zoonotic disease control and subnational surveillance. This demonstrates a key operational tenant for One Health: strengthening the specialized capacity of each sector while providing pathways to bring information and action together in coordinated fashion.

The applied format of the one-month course at multiple geographic scales allowed participants to identify practical opportunities to overcome One Health implementation barriers. Among these are: 1) the need for synchronization of standard operating procedures for zoonotic diseases for surveillance and pathogen detection, 2) improved networking between field and laboratory teams, and 3) the establishment of feedback mechanisms to communities to facilitate their engagement in disease reporting and control. Applied and in-depth trainings such as the PREDICT workshop help strengthen field-based skills and enhance awareness of pathways for integration of zoonotic disease prevention, detection, and response measures in the surveillance system.

PARTNERS: Sokoine University of Agriculture, Ifakara Health Institute, UC Davis, USAID PREDICT-2
GHSA: PREVENT-2: Zoonotic Disease; DETECT-5: Workforce Development

PRIORITIZING & OPERATIONALIZING RISK REDUCTION

VIET NAM

Viet Nam has been a leader in advancing One Health in practice since 2006 through development of coordinated approaches to tackle Highly Pathogenic Avian Influenza and in its current role as a co-lead for the Global Health Security Agenda (GHSA) Zoonotic Diseases Action Package. In 2016 the country established an updated 5-year One Health Strategic Plan for zoonotic diseases, which integrates One Health across more than 20 national sectoral and topical plans to strengthen capacity, enhance preparedness, and reduce impact of priority zoonoses and antimicrobial resistance. The plan's six main focus areas include a dedicated item on prevention of infectious disease emergence (Focus Area 3: One Health approaches for managing zoonotic agents with pandemic potential that are yet to emerge), with specific outcome indicators on: 1) Identification of risk factors or interfaces associated with spill-over, amplification and/or spread, and 2) Implementation of risk reduction strategies based on identified risk factors. The plan also recognizes the need for environmental health research on disease drivers (for example, on land use practices) and greater engagement overall of the environment sector through guidelines, policies, and resource allocation to support One Health capacity building.

WHAT ONE HEALTH ADDED

To date, the emphasis for novel disease outbreaks has been on mobilizing effective and rapid response to avoid international disease spread. Viet Nam's application of One Health promotes a preventive stance that moves upstream to target pathogen spillover at the source to avoid or reduce human and animal disease and their associated health and economic consequences. The plan has been made operational through initial activities at key interfaces where wildlife-human exposure is common and presents potential zoonotic disease transmission pathways (e.g. live animal markets, bat guano collection sites, and wildlife restaurants). PREDICT has been a key partner in strengthening capacity for sample collection and screening for novel pathogens, addressing key challenges identified in the plan, including workforce development for risk monitoring and mitigation at high-risk animal-human interfaces (wildlife farms, bat guano harvest sites, and rodent trade routes) through on-the-job training for Dong Nai Province Forest Protection Department, the Regional Animal Health Office No. 6 & 7, and National Institute of Hygiene and Epidemiology staff who participated in PREDICT surveillance planning, sample collection, and diagnostic activities. With PREDICT's support, all partner laboratories, including national public health and animal health laboratories in Viet Nam, are trained in the full range of activities required for safely detecting zoonotic viruses, such as biosafety and biosecurity, cold chain, safe sample storage, data management, safe sample transport and shipping, and molecular virus detection techniques.

The country has also recognized the need for multisectoral information sharing to promote biodiversity and health mainstreaming across a range of stakeholders. For example, PREDICT shared results and lessons on human health risks associated with the illegal wildlife trade at a United Nations Office on Drugs and Crime (UNODC) training on the Penal Code in July 2018, helping to strengthen awareness about a potential driver of disease emergence in addition to biodiversity loss. Viet Nam's national commitment to reducing the threat of novel pathogens is a model for all countries to get ahead of disease emergence events, which is adaptable to high-risk species and exposure interfaces relevant to each country or community.

PARTNERS:	Wildlife Conservation Society; Department of Animal Health, Ministry of Agriculture and Rural Development; National Institute of Hygiene and Epidemiology, Ministry of Health; Viet Nam National University of Agriculture; Regional Animal Health Office No. 6 & 7; Dong Nai Province Forest Protection Department; PREDICT-2
GHSA:	PREVENT-2: Zoonotic Disease; DETECT-5: Workforce Development; RESPOND-2: Linking Public Health with Law and Multisectoral Rapid Response

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