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**REDUCING PANDEMIC RISK,
PROMOTING GLOBAL HEALTH**



REDUCING PANDEMIC RISK, PROMOTING GLOBAL HEALTH

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REDUCING PANDEMIC RISK, PROMOTING GLOBAL HEALTH

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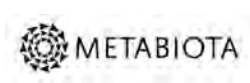
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“Infectious diseases continue to be a serious burden around the world, in developing and industrialized countries alike. Whether naturally occurring or intentionally inflicted, microbial agents can cause illness, disability, and death in individuals while disrupting entire populations, economies, and governments. In the highly interconnected and readily traversed “ global village” of our time, one nation’ s problem soon becomes every nation’s problem as geographical and political boundaries offer trivial impediments to such threats. The United States has shown leadership in the past by strengthening its own and others’ capacities to deal with infectious diseases, but the present reality is that the public health, veterinary, and medical-care communities are inadequately prepared. We must do more to improve our ability to prevent, detect, and control microbial threats to health.”

– INSTITUTE OF MEDICINE, MICROBIAL THREATS TO HEALTH

TABLE OF CONTENTS

Abbreviations and Acronyms	6
Executive Summary.....	8
Introduction.....	16
Major Global Achievements.....	22
Surveillance Strategy	30
Virus Detection and Discovery	40
Information Management.....	48
Capacity Building.....	64
Outbreak Response	72
Operationalizing One Health.....	78
Influenza	84
Deep Forest.....	90
Modeling and Analytics.....	96
Highlights of PREDICT Publications.....	118
Future Directions	150
Bangladesh.....	156
Bolivia	164
Brazil	176
Cambodia	186
Cameroon.....	196
China	210
Democratic Republic of Congo	220
Gabon	232
Indonesia.....	240
Lao PDR	248
Malaysia	258
Mexico	268
Nepal.....	278
Peru.....	286
Republic of Congo.....	294
Rwanda.....	302
Tanzania.....	310
Thailand.....	320
Uganda	328
Vietnam	338
PREDICT Consortium Management.....	348


ABBREVIATIONS AND ACRONYMS

BASV	Bas-Congo virus
BSL	Biohazard safety level
CBD	Convention on Biological Diversity
CCHF	Crimean-Congo hemorrhagic fever
CDC	United States Centers for Disease Control and Prevention
CII	Center for Infection and Immunity at Columbia University
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CoV	Coronavirus
cPCR	Consensus polymerase chain reaction
DNA	Deoxyribonucleic acid
DRC	Democratic Republic of Congo
DUO	Disease of unknown origin
EHA	EcoHealth Alliance
EID	Emerging infectious disease
EMCV	Encephalomyocarditis virus
EPT	Emerging Pandemic Threats Program of USAID
EVD	Ebola virus disease
FAO	Food and Agricultural Organization of the United Nations
FUO	Fever of Unknown Origin
GAINS	Global Animal Information System
GDCDC	Centers for Disease Control of Guangdong Province
GDIPH	Guangdong Institute of Public Health
GDP	Gross domestic product
GIS	Geographical information system
GPS	Global Positioning System
GRID	Global Repository of Infectious Diseases
HALI	Health for Animals and Livelihood Improvement
HIV/AIDS	Human immunodeficiency virus/acquired immune deficiency syndrome
HPAI	Highly pathogenic avian influenza
HPS	Hantavirus pulmonary syndrome
HTS	High through-put sequencing
ICTV	International Committee on Taxonomy of Viruses
IDRC	International Development Research Centre
ILI	Influenza-like Illness
IUCN	International Union for Conservation of Nature
Lao PDR	Lao People's Democratic Republic
LDI	Landscape Disturbance Index

MB	Metabiota
MERS	Middle East Respiratory Syndrome
MGVP	Mountain Gorilla Veterinary Project
NBCA	National Biodiversity Conservation Area
NGO	Non-governmental organization
NIH	National Institute of Health
OHASA	One Health Alliance South Asia
OHCEA	One Health Central and Eastern Africa
OHI	One Health Institute
OIE	Office International des Epizooties (World Organization for Animal Health)
PAHO	Pan-American Health Organization
PPE	Personal protective equipment
RNA	Ribonucleic acid
ROC	Republic of Congo
RT-PCR	Reverse transcription polymerase chain reaction
SARI	Severe Acute Respiratory Infection
SARS	Severe acute respiratory syndrome
SI	Smithsonian Institution
SIV	Simian immunodeficiency virus
SSC	Species Survival Commission
STLV-4	Simian T-lymphotropic virus type 4
SURPI	Sequence-based ultrarapid pathogen identification
UCD	University of California, Davis
UNEP	United Nations Environment Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
USA	United States of America
USAID	United States Agency for International Development
USD	United States dollar
VHF	Viral hemorrhagic fever
WCS	Wildlife Conservation Society
WHO	World Health Organization
WNV	West Nile virus
WWF	World Wide Fund for Nature
YFV	Yellow fever virus

EXECUTIVE SUMMARY





The appearance and spread of diseases, such as HIV/AIDS, Severe Acute Respiratory Syndrome (SARS), Ebola virus disease (EVD), and pandemic influenza, have had profound global health impacts and adverse ramifications for human livelihoods and broader scale economics. The lives lost and financial consequences have illustrated our vulnerability to the emergence and re-emergence of infectious diseases and the disappearing boundaries between the developing and developed world.

Zoonotic diseases caused by pathogens that are shared between people and animals result in millions of deaths annually, and the economic losses from a single outbreak can amount to tens of billions of dollars. Prevention and early control of outbreaks is key to reducing the impact of epidemics and pandemics, but there remains a critical need to improve global capacity to effectively implement these mitigating activities, especially in developing countries, as demonstrated by the 2014 EVD epidemic in West Africa.

Despite greater recognition of emerging infectious diseases (EIDs), there is limited understanding of the underlying causes for emergence and spread of zoonotic pathogens in people. Viral diseases originating from wildlife have been responsible for most zoonotic EIDs in recent history. The seriousness of the risks associated with viral spillover from animals to people has led to the recognition that a shift from a conventional, reactive approach (once a disease is spreading in human populations) toward a proactive, predictive approach is necessary for EID prevention and timely control (Figure 1). The general lack of specific medicines and vaccines for new zoonotic viruses puts a premium on developing non-pharmaceutical interventions based on a detailed understanding of when, where, and how zoonotic viruses are moving from wildlife to people.

The United States Agency for International Development (USAID) initiated the Emerging Pandemic Threats (EPT) program in 2009 with the goal of strengthening capacities in developing countries to prevent, detect, and control infectious diseases. PREDICT, a surveillance and virus discovery component of the EPT program, focused on building capacity to identify potential zoonotic viral threats at high-risk wildlife-human pathogen transmission interfaces where diseases are most likely to emerge. These interfaces occur in many cultural contexts and regions and usually result from necessary daily activities, such as animal-based food production/acquisition and other income-generating activities, such as preparation or harvesting guano for agricultural fertilizer from structures constructed to attract bats (photo on previous page). Through a consortium of global and in-country partners, PREDICT's efforts focused on early detection and response to potentially high-consequence animal viruses in regional "hotspots" for EIDs, such as central Africa, South and Southeast Asia, and Latin America. The consortium developed robust mechanisms for overcoming geographic and disciplinary constraints to public health protection by developing multidisciplinary collaborations and establishing networks and platforms for surveillance, diagnostics, and data sharing and interpretation across stakeholders.

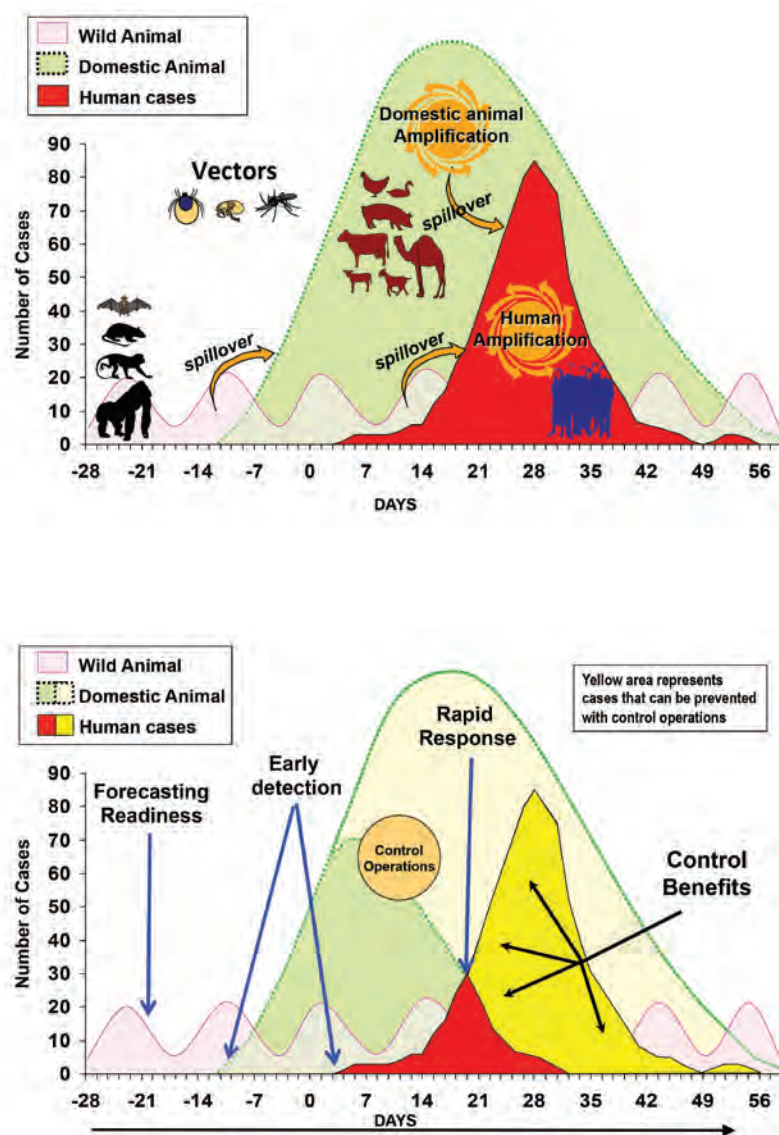


Figure 1. (Top) Transmission to and amplification of zoonotic pathogens in people (red) occurs after a virus from wild animals (pink) spills over into livestock (green) or people to cause an outbreak. Spillover arrows illustrate cross-species transmission.

(Bottom) Forecasting and early detection and control efforts reduce disease incidence in animals (green) and people (red). Adapted from Karesh et al. 2012.

PREDICT, implemented in over 20 countries, improved early detection and response to disease threats through five main objectives: 1) strengthening viral surveillance; 2) improving virus detection and discovery by developing laboratory and disease outbreak response capacities; 3) characterizing high-risk animal-human interfaces, behaviors, and drivers of pathogen spillover from animals to people; 4) optimizing predictive models for disease emergence and spread; and 5) deploying cutting-edge information management and communication tools to advance a more integrated, global approach to sharing data from zoonotic virus surveillance.

By enhancing in-country capacity for detection, response, and prevention of pathogen spillover and increasing connectivity among government sectors (wildlife, livestock, and human health), PREDICT effectively engaged 59 government ministries and hundreds of scientific institutions, local organizations, and other stakeholders to significantly advance One Health capacity. Our team trained 2,500 government personnel, physicians, veterinarians, resource managers, laboratory technicians, hunters, and students on biosafety, surveillance, laboratory techniques, and disease outbreak investigation. With USAID support, we equipped, supplied, and trained staff in 32 diagnostic laboratories around the world to safely and properly process and test wildlife samples for viral pathogens of known and unknown zoonotic potential. This capacity-building effort led to the safe and humane sampling of more than 56,340 nonhuman primates, bats, rodents, and other wild animals (including samples from bushmeat). PREDICT detected 959 viruses in wild animals and 34 viruses in human pilot studies (some viruses detected in both humans and animals). Viruses were classified as novel (genetically divergent from known strains and species) or known (sufficiently similar to known viruses) based on genetic sequencing. Thus far, samples have yielded 815 novel viruses and 169 known viruses.

PREDICT

THE WORLD'S MOST COMPREHENSIVE
ZOO NOTIC DISEASE SURVEILLANCE &
CAPACITY DEVELOPMENT PROGRAM

TRAINED 2,500 government personnel, physicians, veterinarians, resource managers, laboratory technicians, hunters, and students on biosafety, surveillance, lab techniques, and disease outbreak investigation.



SAMPLED 56,000+ nonhuman primates, bats, rodents, and other wild animals (including bushmeat samples) at human-wildlife interfaces with high-risk and opportunity for viral spillover from wildlife hosts to humans.



DEVELOPED & OPTIMIZED low-cost viral family level consensus PCR methods and synthetic controls for the detection and discovery of new viruses from the target viral families in 32 labs in 20 developing countries around the world.



DETECTED a total of 984 unique viruses in wild animals and humans: 815 novel viruses and 169 known viruses – the most comprehensive viral detection and discovery effort to date.



handling poultry. More broadly, high-risk interfaces reported in zoonotic disease transmission of viruses from wildlife to humans in the literature include contact with wildlife in and around human dwellings and during hunting or consumption of wildlife, in addition to occupational exposures including veterinarians; researchers; and workers in laboratory settings, agricultural fields, wildlife management, zoos, and sanctuaries (Figure 2). These high-risk interfaces are important targets for pathogen surveillance and may be critical points for implementation of disease prevention and control measures. Other interfaces were also targeted by surveillance to more fully investigate and rank risks for potential virus transmission, including wild animal farms; markets and restaurants; other sites on the food value chain; sites with ecotourism; and wildlife preying on livestock, raiding crops, and causing public safety hazard.

While PREDICT surveillance activities were not designed to specifically target influenza A viruses, our diagnostic strategy did include protocols to detect influenza viruses. Thus PREDICT contributed protocols for testing to laboratories and facilitated influenza A screening of wildlife and human samples in collaborating laboratories. In order to understand the role wild birds play in the emergence of zoonotic influenza viruses, PREDICT scientists examined 11,870 sequences from the National Institutes of Health genetic sequence database, GenBank, and gathered data from 50 studies and over 250,000 birds to provide a baseline inventory and insight into patterns of global influenza A subtype diversity and richness in wild birds. Over 116 influenza A strains occurred in wild birds globally, which is approximately twice the number found in domestic birds. In an effort to understand factors driving the evolution and diversity of all high-risk influenza A virus subtypes and more accurately identify hotspot areas of emergence to better design diagnostic strategies, PREDICT investigators also evaluated mutation rates of high priority influenza A subtypes detected globally as well as socio-economic, biodiversity, and agricultural drivers that may be associated with subtype diversity and reassortment. Results indicated that potentially pathogenic influenza A strains may be more likely to evolve in East Asia, reinforced by the fact that the majority of subtypes that have caused disease and mortality in humans in recent years, including H5N1 and H7N9, were first detected in China and Hong Kong. In addition, PREDICT researchers assisted partners in China and 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 research revealed that the H7N9 outbreak lineage originated from reassortment of H7 viruses and enzootic H9N2 viruses and that the H7 viruses likely transmitted from domestic ducks to chickens in China during two separate events. An important recommendation for diagnostic testing was to revise current strategies of targeted surveillance for specific influenza subtypes – instead performing broader testing to detect all subtypes in order to better understand the total diversity globally and to facilitate the early detection of emerging subtypes and strains.

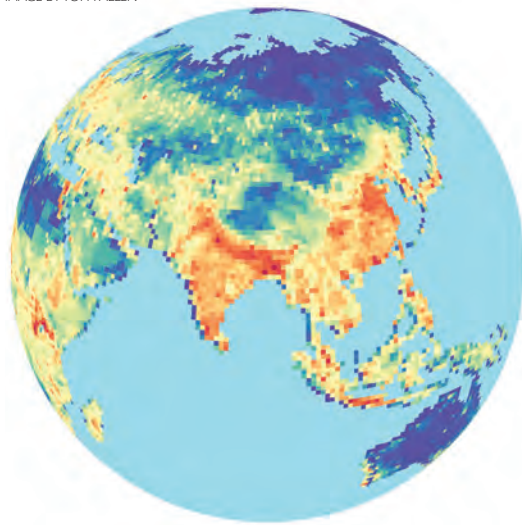
Transporting poultry by motorbike, Guangxi, China.



PHOTO BY PETER DASZAK, EHA

IMAGE BY TOPH ALLEN

**New zoonotic
disease emergence
forecasting model –
Hotspots II**



PREDICT improved our understanding of the underlying causes for disease emergence by building upon previous research to assess temporal and spatial patterns of disease emergence globally. The newly developed risk map (Hotspots II depicted at left) focuses on emerging zoonotic viruses from wildlife and provides a more refined projection of EID risk globally. The new Hotspots II model independently confirmed that the risk for zoonotic disease emergence increases with higher mammal diversity. Land-use type and land-use change are the other most important factors predicting emergence of zoonotic diseases of wildlife-origin.

Our scientists also implemented the Deep Forest (DF) study to further enhance the understanding of ecological factors that drive zoonotic viral disease emergence due to land-use change. This work has and will continue to refine our approach in the global scale “hotspots” modeling, by providing detailed information about risk at a local level – a scale at which humans live and interact with wildlife and livestock. Characterizing known and unknown viral diversity and describing the relationship between viral diversity, host diversity, land-use change, and human ecology are critical for better understanding of the ecological processes behind zoonotic disease emergence so that disease outbreaks can be prevented or their impacts minimized. Mathematical modeling used in PREDICT has expanded knowledge of viral traits, host species, and high-risk interfaces as predictors of susceptible host taxonomic range, human-to-human transmission, and geographic spread of viruses.

Interpreting and sharing of information with the host governments in PREDICT countries highlighted the critical steps of data collection, management, validation, and verification that are often overlooked. All data were carefully examined upon integration into the purposefully-designed internal information management system. When test results (e.g. initial detection and subsequent sequence confirmation of viruses) were produced for a given specimen, the data were interpreted in light of all available scientific literature by PREDICT virologists. This iterative process ensured the highest quality, most robust data possible. After interpretation, results were provided to host governments for examination, used to inform policy, and approved for public sharing. Once cleared for release by host country governments, our surveillance data were integrated with HealthMap’s digital surveillance data and spatial information generated from PREDICT’s disease hotspots modeling and shared with the public on the PREDICT data site (www.healthmap.org/predict). The open access, online platform provides users with a tool to visualize PREDICT surveillance along with disease events worldwide. All viral genetic sequences obtained during the project are also being deposited in the GenBank database for public access.

The PREDICT consortium designed and implemented a targeted risk-based surveillance strategy as an approach to pandemic prevention based not on humans as sentinels of disease but on detecting viruses early, at their source, where intervention strategies can be implemented before there is opportunity for spillover and spread in people. As a testament to the degree to which PREDICT and its partners have truly advanced wildlife surveillance and supported scientific

excellence and transparent communications, in-country staff and partners have been requested to serve on national disease task forces and to provide technical and expert assistance for several high-profile disease outbreak investigation and response efforts, including H7N9 influenza A, Nipah virus, MERS, and multiple EVD outbreaks. PREDICT's success in building local capacity to detect hundreds of viruses in wild animals, coupled with a series of ongoing cutting-edge modeling and analytical activities evaluating the risk of emerging viral zoonoses, have significantly improved the world's baseline knowledge on the zoonotic pool of viruses and the risk of exposure to people. Further testament to the success and utility of the PREDICT project and the work of its team of dedicated One Health professionals are the resulting 90 peer-reviewed, scientific publications that improve our understanding of zoonoses and the factors influencing their emergence. The wide distribution of the project findings from these scientific publications is assisting in cutting-edge global health improvements, including surveillance science, diagnostic technologies, understanding of viral evolution, and ecological driver identification. Through its One Health leadership and highly productive partnerships, PREDICT has forged new networks of professionals from the human, animal, and environmental health sectors to promote global health, improving our understanding of high-risk interfaces for viral disease spillover from wildlife into people and implementing strategies for preventing and controlling emerging disease threats. In the next phase, the PREDICT consortium will focus on ranking risk of spillover, amplification, and spread of these newly detected viruses using all available virological, epidemiological, ecological, and host-specific data. A targeted approach will be used to identify risky human behaviors for transmission of zoonotic viruses, and potential hosts (animal and human) at high-risk interfaces will be evaluated in concert to document pathogen sharing. This approach will build on the findings and capacity built during the first phase of PREDICT to better target effective detection, diagnosis, intervention, and prevention of EIDs of pandemic potential.

INTRODUCTION



Emerging infectious diseases (EIDs) pose substantial threats to the health of animals and people and economies globally. Over the past several decades, the emergence and spread of viruses such as severe acute respiratory syndrome (SARS) coronavirus, Ebola virus, and H5N1 and H1N1 pandemic influenza A viruses, have had profound global health and economic impacts, illustrating our vulnerability to the emergence and re-emergence of zoonotic diseases.

Zoonotic diseases result in millions of deaths annually, and the economic losses from a single outbreak can amount to tens of billions of dollars (e.g. SARS; Lee and McKibbin 2004; Keusch et al. 2009; World Bank 2012). Prevention and early control of outbreaks is key to reducing their impact, but there remains a critical need to improve the capacity of developing countries to effectively implement prevention and control activities as demonstrated by the 2014 Ebola virus disease outbreak in West Africa.

Zoonotic pathogens shared with wildlife or livestock account for the majority of emerging infectious diseases of people, and viruses comprise 25-44% of these emerging and re-emerging pathogens (Jones et al. 2008; Taylor et al. 2001). Because of their diversity and rapid evolution, viruses, in particular RNA viruses, are perceived to pose the greatest global pandemic threat (Morse 1995; Cleaveland et al. 2001; Pulliam 2008). For instance, two of the most devastating pandemics in human history, the 1918 influenza pandemic (50 million deaths) and the HIV/AIDS pandemic (35 million deaths as of 2012; Morens et al. 2008; Fauci and Folkers 2012), were caused by zoonotic RNA viruses.

Despite greater recognition of EIDs on a global scale, there is limited understanding of the underlying causes for emergence and spread of zoonotic pathogens in people (Murray and Daszak 2013). Factors associated with emergence, re-emergence, and spread of pathogens have been categorized into four broad domains worthy of investigation: 1) genetic and biological factors of pathogens and hosts, such as microbial adaptation and human susceptibility to infection; 2) physical environmental factors (e.g. climate variability and weather); 3) ecological factors (e.g. land-use change); and 4) social, political, and economic factors, including poverty and animal and public health infrastructure (Bogich et al. 2012; Daszak et al. 2001; Smolinski et al. 2003). For example, factors such as rapid human population growth, land use change, intensification of livestock production, natural resource extraction, and

Indochinese silvered langur (*Trachypithecus germaini*) kept as a pet alongside domestic dogs in Cambodia.



PHOTO BY WILDLIFE CONSERVATION SOCIETY

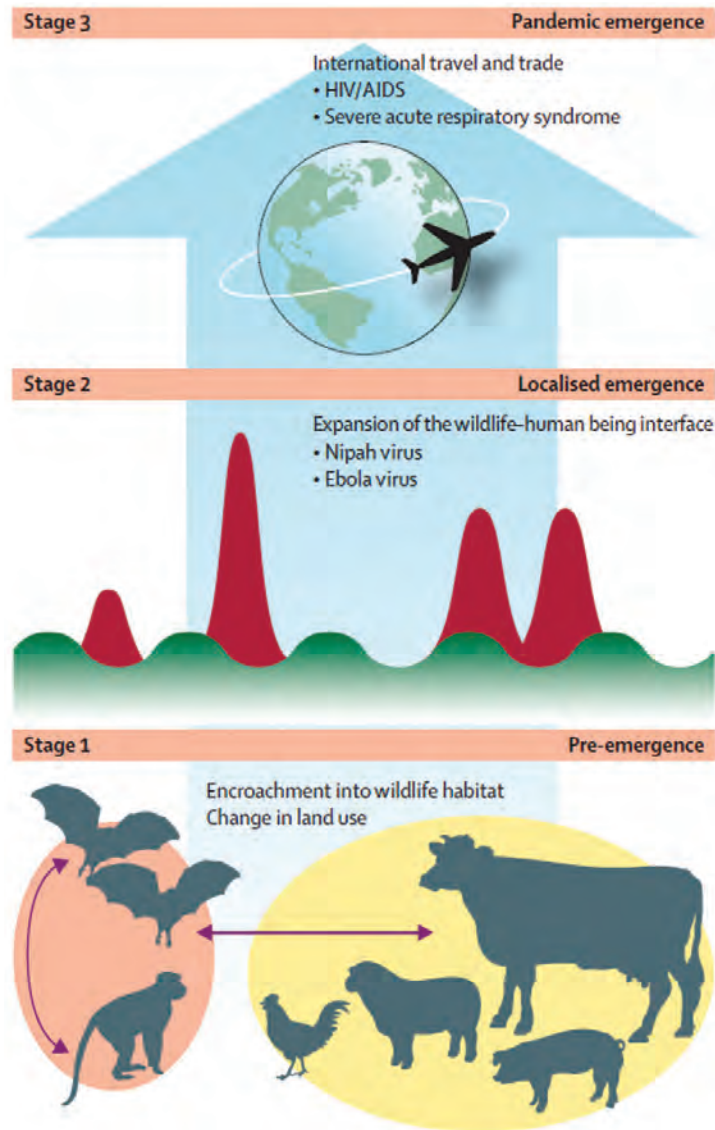


Figure 1: Emergence of pandemic zoonotic disease. From Morse et al. 2012.

Stage 1 is a pre-emergence state, in which microbes are transmitted between animal reservoirs. Ecological disturbances (e.g. due to land use change) modify the dynamics of microbial transmission, which can lead to a heightened risk of pathogen spillover to wildlife or livestock hosts.

Stage 2 is localized emergence, either through self-limiting spillover events (green peaks and troughs, representing increases and decreases in the numbers of infected people with time) or large-scale spillover (red peaks, representing spikes in the number of infected people with time), that leads to person-to-person transmission for a few pathogen generations.

In stage 3, some spillover events might lead to sustained person-to-person outbreaks, international or global spread, and the emergence of a pandemic. The size, spread, and potential effect of events increase from stage 1 to stage 3, but the frequency falls so that full stage 3 pandemics are quite rare. By dissecting this process and analyzing the interactions of the underlying drivers with the risk of spillover and spread, development of a more structured approach to pandemic prevention is possible. The ultimate goal of successful pandemic prevention is to target control at stage 1.

the demands for global trade result in greater interactions between animals and people and therefore more opportunities for cross species transmission and disease emergence (Daszak et al. 2001; Smolinski et al. 2003). International travel has also contributed to the spread of many infectious diseases regionally or globally with one of the most notable being the spread of SARS coronavirus from Hong Kong to North America, Latin America, Europe, Asia, and Australia resulting in 8,422 documented cases and 916 deaths worldwide (Heymann and Rodier 2004).

Wildlife play a major role in the emergence of infectious diseases in human populations. Analysis of over 300 EID events occurring between 1940 and 2004 revealed that more than 70% of emerging zoonotic pathogens originated in wildlife, and that the incidence of EID events caused by wildlife pathogens has significantly increased over time (Jones et al. 2008). Certain wildlife taxa are known to harbor a high proportion of zoonotic pathogens. Among assessed wildlife taxa, zoonotic viruses have been most commonly detected in rodents, bats, primates, ungulates, and birds (Woolhouse and Gowtage-Sequeria 2005; Calisher et al. 2006; Luis et al. 2013; Smith and Wang 2013). Therefore, focusing surveillance efforts on these wildlife hosts is likely to enhance the identification of viruses with pathogenic and disease emergence potential prior to spillover to people (Parrish et al. 2008; Morse et al. 2012). Efforts in this realm must also be cognizant of the need for clear messaging to not vilify wildlife, as the mechanisms for disease spillover tend to be primarily human-induced, not animal-driven, and biodiversity and conservation goals must also be maintained for a healthy world.

The seriousness of these threats has led to the recognition that a shift from a conventional, reactive approach toward a proactive, predictive approach is necessary for EID prevention and timely control (Figure 1; Karesh et al. 2012; Morse et al. 2012). Infrastructure improvements and technological advances have dramatically and rapidly improved our ability to identify high-risk interfaces for disease transmission and to detect novel pathogens before widespread spillover occurs. These advances include improvements in information technology, molecular diagnostics, and risk modeling. The challenge lies in developing a strategic framework to identify zoonotic pathogens of pandemic potential that have not yet emerged in human populations.

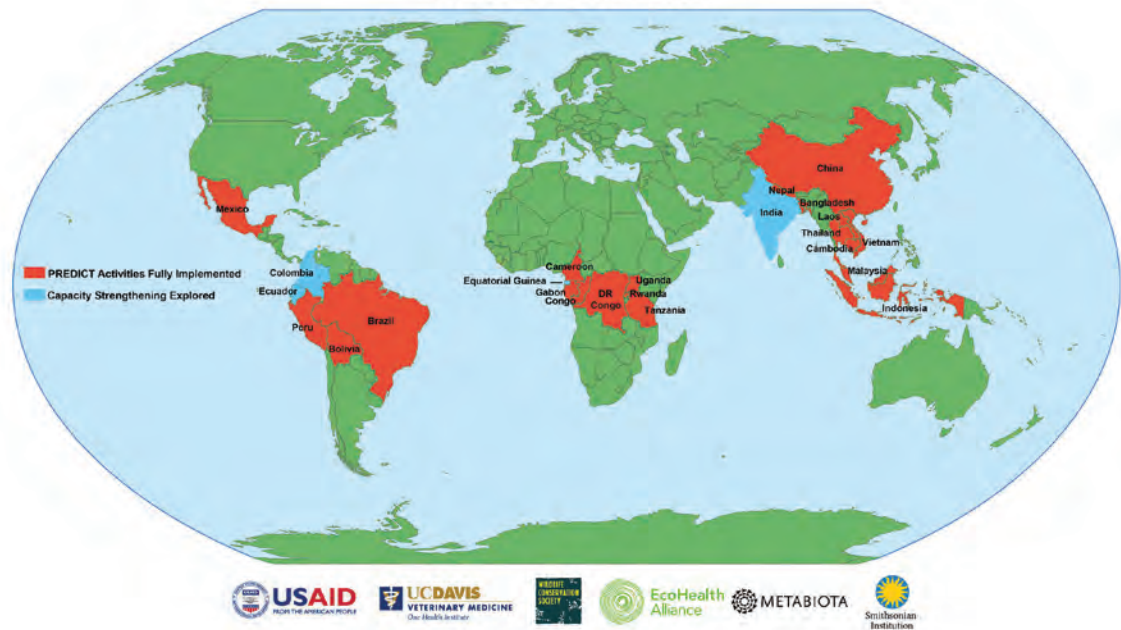
To this end, USAID initiated the Emerging Pandemic Threats (EPT) program in 2009. The initial goal of the EPT program was to strengthen capacities in developing countries to prevent, detect, and control infectious diseases. Efforts were focused on early detection and response to potentially high-consequence animal viruses before they became significant public health threats in regional “hotspots” for emerging diseases, such as central Africa, South and Southeast Asia, and Latin America (USAID 2013).

Rodents being sold for consumption in an African market.



PHOTO BY PIOTR NASKRECKI

PREDICT Countries



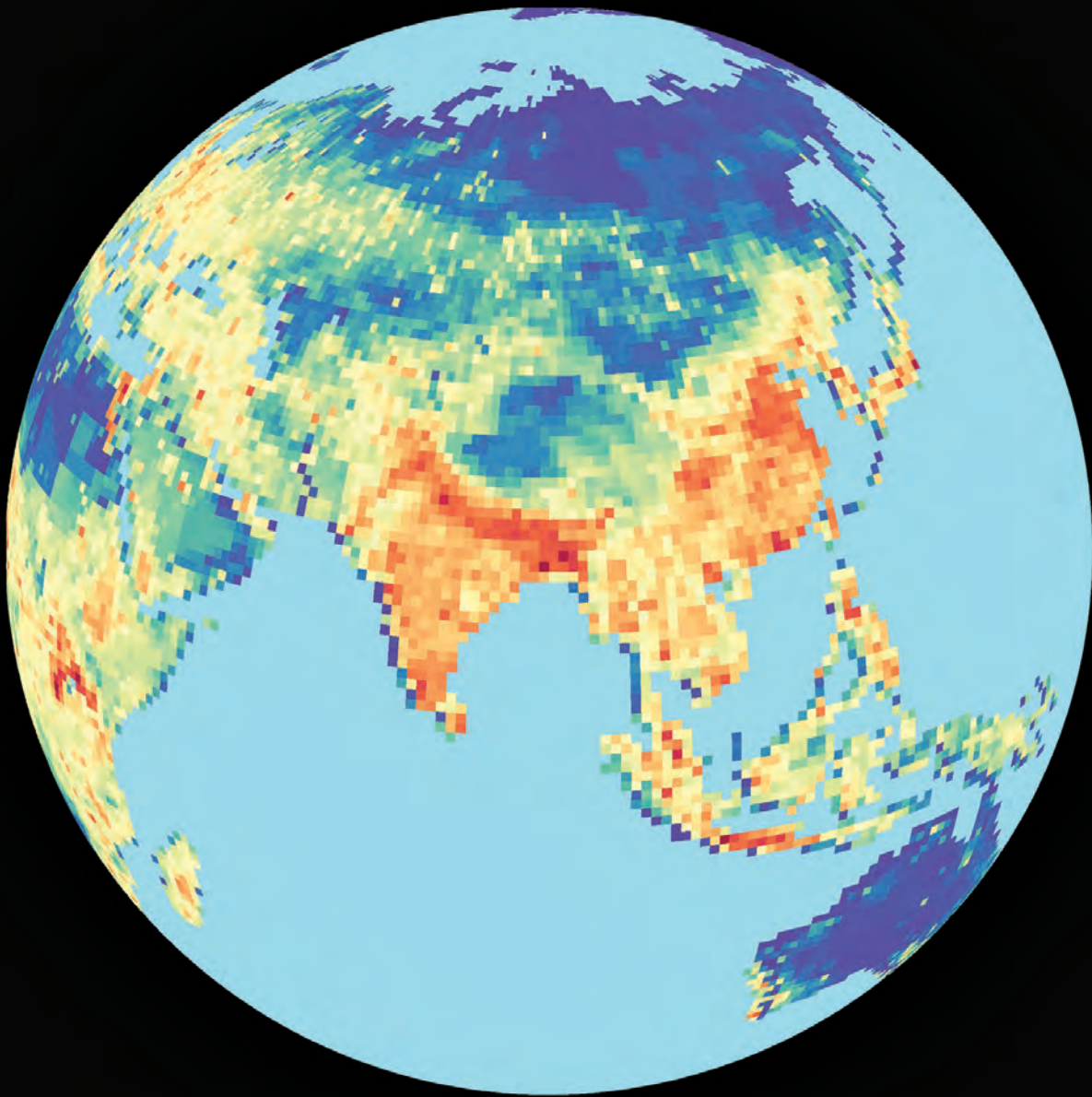
PREDICT, a surveillance and virus discovery component of the EPT program, focused on building capacity to identify potential zoonotic disease threats at high-risk wildlife-human disease transmission interfaces where diseases are most likely to emerge. PREDICT was implemented in more than 20 countries, including Bangladesh, Bolivia, Brazil, Cambodia, Cameroon, China, Democratic Republic of Congo, Gabon, Indonesia, Laos, Malaysia, Mexico, Nepal, Peru, Republic of Congo, Rwanda, Tanzania, Thailand, Uganda, and Vietnam. The program aimed to improve early detection and response to disease threats through five main objectives: 1) strengthening disease surveillance systems; 2) improving viral discovery by developing laboratory and disease outbreak response capacity; 3) characterizing drivers of pathogen spillover and spread from animals to people; 4) optimizing predictive models for disease emergence and spread, and 5) deploying cutting-edge information management and communication tools to advance a more integrated, global approach to emerging zoonotic diseases.

Through EPT, PREDICT worked to enhance in-country capacity for response and prevention of pathogen spillovers by strengthening laboratory and diagnostic systems and increasing connectivity among government sectors (wildlife, livestock, and human health) to operationalize a One Health platform and promote early detection. In addition, PREDICT conducted surveillance and monitoring for viruses in wildlife at high-risk animal-human interfaces to detect potential zoonotic threats and prevent their emergence in human or livestock populations.

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MAJOR GLOBAL ACHIEVEMENTS



PREDICT conducted the most comprehensive zoonotic disease surveillance capacity development program in the world, unprecedented in scope and productivity. The PREDICT consortium, led by the UC Davis One Health Institute, EcoHealth Alliance, Metabiota, the Wildlife Conservation Society, and the Smithsonian Institution, developed and stewarded the five year project starting in 2009.

PREDICT was implemented in Africa (Cameroon, Gabon, Republic of Congo, Democratic Republic of Congo, Tanzania, Uganda, and Rwanda), Asia (Bangladesh, Cambodia, China, Indonesia, Laos, Malaysia, Nepal, Thailand, Vietnam), and Latin America (Bolivia, Brazil, Mexico, and Peru) as part of USAID's global Emerging Pandemic Threats (EPT) program.

DESIGNED AND IMPLEMENTED HIGHLY TARGETED RISK-BASED SURVEILLANCE

PREDICT designed and implemented a highly targeted risk-based surveillance strategy as an approach to pandemic prevention based not on humans as sentinels of disease but on detecting viruses early at their source where intervention strategies can be implemented before there is opportunity for spillover and spread in people. To discover potentially pathogenic viruses before they emerge, surveillance efforts were focused on wildlife taxa most likely to serve as reservoirs for emerging zoonotic viruses (i.e. bats, rodents, and nonhuman primates) at human-animal interfaces with high-risk potential for disease transmission (see Surveillance Strategy section). High-risk interfaces are situations where people have frequent, direct, and often intense contact with these wild animals, such as when they are hunted for food or trade and where wildlife raid crops, share water sources, or enter and live in human dwellings. In addition, land-use changes were assessed in the context of how they could modify the risk of pathogen spillover by perturbing the dynamics of viruses in wildlife and/or by bringing naive animal species (including humans) into close contact. Samples specific to human-wildlife contact and likely routes of disease transmission were collected and tested for viruses related to genera or taxonomic families known to cause epidemics and pandemics in people to maximize detection of viruses of major public health importance.

PREDICT trained local project staff and their colleagues, as well as university and government partners, to safely and humanely sample more than 56,340 nonhuman primates, bats, rodents, and other wild animals (including from bushmeat) at animal-human interfaces with heightened opportunity for viral spillover from wildlife hosts to humans. To further assess human risk at these high-risk interfaces, PREDICT piloted a number of human disease surveillance efforts in Uganda, the Democratic Republic of Congo, Cameroon, Gabon, Malaysia, China, and Indonesia.



PHOTO BY GORILLA DOCTORS

Dr. Olivier Nsengimana, Field Veterinarian, PREDICT Rwanda, processes wildlife samples in the wildlife virology laboratory at the Rwanda Agriculture Board in Rubilzi, Kigali, Rwanda.

DEVELOPED AND OPTIMIZED INNOVATIVE DIAGNOSTIC TESTING METHODS IN DEVELOPING COUNTRIES

PREDICT developed and optimized low-cost viral family-level consensus PCR methods and synthetic controls for the detection and discovery of known and new viruses. The protocols were implemented in 32 labs in the 20 countries selected by USAID for the EPT program. Uptake of these methods by in-country partners has built long-term capacity and partnerships for identifying known and novel viruses of animal and human origin.

DISCOVERED VIRUSES AT HIGH-RISK INTERFACES AND ENHANCED OUR UNDERSTANDING OF DRIVERS OF DISEASE EMERGENCE

PREDICT's success in building local capacity to detect hundreds of viruses in wild animals, coupled with a series of on-going cutting-edge modeling and analytical activities evaluating the risk of emerging viral zoonoses, have significantly improved the world's baseline knowledge on the pool of zoonotic viruses and the risk of exposure to people.

Since 2009, PREDICT has detected a total of 984 unique new and known viruses in wild animals and humans in regions where diseases are mostly likely to emerge (Table 1; see Virus Detection and Discovery section for breakdown of viruses detected by viral family).

Table 1. PREDICT viruses detected to date by region.

	Number of Viruses Detected in Animals and Humans*
Asia	631
Africa	420
Latin America	73

*Note: numbers of viruses do not total to 984, as some viruses have been found in more than one region.

All viral genetic sequences are being deposited in the GenBank database for public access. Analyses incorporating all virus detections (i.e. all virus families combined) globally for PREDICT to date validate our surveillance strategy. For example, results show that PREDICT's target wildlife taxa (i.e. bats, nonhuman primates, and rodents) were significantly more likely to test positive for a virus than other taxonomic groups sampled (see Figure 1 for proportion of positive test results by animal host taxonomic groups sampled).

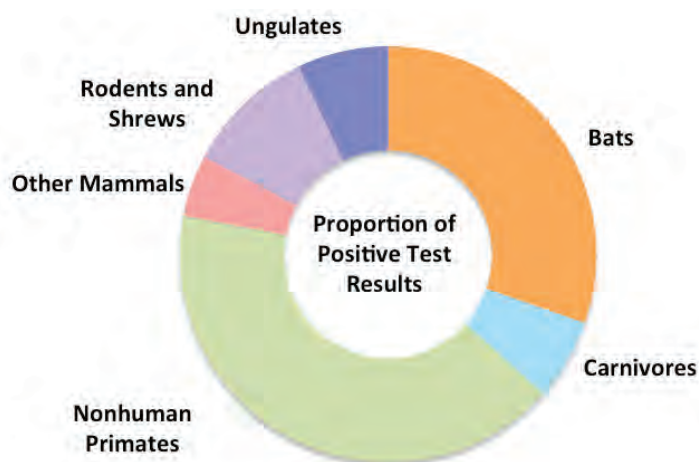


Figure 1. Proportion of positive test results for viruses detected to date among animal host taxonomic group sampled.

Among the 6,787 positive test results (out of 419,919 tests conducted to date), PREDICT detected 959 unique viruses in wild animals (in addition to the 34 viruses detected in human pilot studies). Viruses were classified as novel (genetically divergent from known strains and species) or known (sufficiently similar to known viruses) based on genetic sequencing. Thus far, our samples from animals have yielded 812 novel viruses (85%) and 147 known viruses (15%).



A member of the PREDICT Tanzania team captures a primate for sampling.

PHOTO BY LIZ VANWORMER

Among positive test results, approximately 58% were positive for novel viruses and 42% were positive for known viruses. Pilot work on samples collected from humans for disease surveillance in Uganda, the Democratic Republic of Congo, Cameroon, Gabon, Malaysia, China, and Indonesia, have so far yielded 31 known and 3 novel viruses (some of the known viruses were also found in animal samples) with testing still on-going (Table 2).

PREDICT's effort in just five years resulted in more viruses detected than the total number of viruses previously recognized in mammals by the International Committee on Taxonomy of Viruses (ICTV). On the list of viruses detected thus far are many important human and animal pathogens such as Severe Acute Respiratory Syndrome (SARS)- and Middle East Respiratory Syndrome (MERS)-related coronaviruses in bats, a novel rhabdovirus (Bas-Congo virus, or BASV) in humans that was associated with acute hemorrhagic fever, and ebolaviruses in humans during multiple outbreaks in Africa. PREDICT is continuing to prioritize which of these viruses should be targeted for further characterization and has been combining these discoveries with data on types and rates of human-wildlife contact in order to assess risk and inform on future surveillance strategies and critical points for implementation of disease prevention and control measures. As part of the viral detection effort, PREDICT developed "viral discovery curves" to estimate the number of undiscovered viruses that exist in a given population, as well as the sample size required to maximize discovery (see Virus Detection and Discovery section). These discovery curves filled a knowledge gap critical for ensuring that surveillance strategies for potential zoonotic threats in wildlife populations are optimal for both viral discovery and cost effectiveness.

Table 2. Viruses detected and discovered to date by taxonomic groups sampled.

	Bats	Nonhuman Primates	Rodents and Shrews	Humans	Other taxa**	TOTAL
Novel	431	234	143	3	9	820
Known	80	55	15	31	1	182
Total						1002*

*Note: numbers of viruses do not total to 984 as viruses have been found in more than one host taxa.

**Other taxonomic groups sampled include carnivores (e.g. bears, civets), lagomorphs (e.g. pikas), pangolins, and ungulates.

Over the span of the project, PREDICT developed and applied a number of innovative modeling and analytical techniques to investigate the risk of emerging infectious diseases, with a focus on emerging zoonoses from wildlife (see Modeling and Analytics section). For example, PREDICT improved our understanding of the underlying causes for disease emergence by building upon previous research to assess temporal and spatial patterns of disease emergence globally. This new model (Hotspots II) focuses on emerging zoonotic viruses from wildlife and provides a more refined projection of EID risk globally.

In addition, PREDICT provided evidence for epidemiologic mechanisms that have long been suspected to promote disease emergence by examining common transmission pathways shared by animal hosts and high-risk activities at transmission interfaces. Mathematical modeling explored viral traits, host species, and high-risk interfaces as predictors of susceptible host taxonomic range, human-to-human transmission, and geographic spread of viruses. Ongoing analyses of the influence of animal-to-human interfaces based on PREDICT test findings by viral family will

also yield information on high-risk behaviors that could be involved in disease transmission for specific viruses detected. In addition, analyses that are underway for the Deep Forest study (see Deep Forest section) will enhance our understanding of the ecological factors that drive zoonotic disease emergence due to land-use change.

ENHANCED ONE HEALTH CAPACITY

PREDICT worked closely with nearly 60 government ministries and hundreds of scientific institutions, local organizations, and other stakeholders to significantly advance One Health capacity and infrastructure in these countries. We trained 2,500 government personnel, physicians, veterinarians, resource managers, laboratory technicians, hunters, and students on biosafety, surveillance, laboratory techniques, and disease outbreak investigation (training materials freely available at: http://www.vetmed.ucdavis.edu/ohi/predict/predict_publications.cfm). With USAID support, the team equipped, supplied, and trained staff in 32 diagnostic laboratories around the world to safely and properly process and test wildlife samples for viral pathogens of known and unknown zoonotic potential.

PREDICT developed robust mechanisms for overcoming geographic and disciplinary constraints to public health protection by developing multidisciplinary collaborations and establishing systems for procurement and data sharing and interpretation across stakeholders. The project actively engaged partners in PREDICT surveillance and diagnostic testing activities. In fact, as a testament to the degree to which PREDICT and its partners truly advanced systems for wildlife surveillance and supported scientific excellence and transparent communications, in-country staff were requested to serve on national disease task forces and to provide technical and expert assistance for several high-profile disease outbreak investigation and response efforts, including H7N9 influenza A, Nipah Virus, MERS, and multiple Ebola virus disease outbreaks.



PHOTO BY MOUNTAIN GORILLA VETERINARY PROJECT

PREDICT conducted surveys in communities surrounding Ebola virus disease and yellow fever outbreaks to better understand types of contact people have with wildlife.

SHARED AND DISSEMINATED INFORMATION

Once cleared for release by host country governments, PREDICT surveillance data were integrated with HealthMap's digital surveillance data and spatial information generated from PREDICT's disease hotspots modeling and shared on the PREDICT public site (www.healthmap.org/predict). The open access, online platform is presented in a user friendly format for a broad audience, providing users with a tool to visualize PREDICT surveillance along with disease events worldwide. As mentioned above, all viral genetic sequences obtained during the project are being deposited in GenBank for public access.

Since 2009, PREDICT staff have been invited to present on the project's approach and findings in some of the world's most respected and powerful institutions and convenings, including multiple presentations at the United States White House, the World Health Organization, the Food and Agriculture Organization of the U.N., the World Organisation for Animal Health, the United States National Academies of Science, and the United States Institute of Medicine. In addition, PREDICT also delivered presentations to the American Society of Microbiology, the Chatham House in London, Prince Mahidol Awards Conference in Thailand, the International One Health Congress, the American Association for the Advancement of Science, International Meeting for Emerging Diseases, International Society for Infectious Diseases, the Food Safety Summit, the American Society of Tropical Medicine and Hygiene, and the American Public Health Association.

PREDICT and the Consortium's many in-country partners, have published more than 90 peer-reviewed, scientific manuscripts that improve our understanding of zoonotic pathogens and drivers of their emergence and that contribute to improvements in surveillance strategies, laboratory methodology, and pathogen discovery and characterization. These manuscripts have been published in *Nature*, the *Proceedings of the National Academies of Science*, *The Lancet*, *Emerging Infectious Diseases*, *mBio*, *PLoS Pathogens*, and numerous other peer-reviewed journals (see Highlights of PREDICT Publications section and http://www.vetmed.ucdavis.edu/ohi/predict/predict_publications.cfm).

IMPROVED OUTBREAK RESPONSE AND PREPAREDNESS

In coordination with USAID EPT partners, PREDICT responded to requests by host governments and other stakeholders to support disease investigations, laboratory diagnostics, supply procurement, and communications in 23 significant outbreaks affecting people, domestic animals, and wildlife, including MERS, H7N9 Influenza, and Ebola virus disease (see Outbreak Response section). PREDICT and EPT partners worked with key stakeholders to operationalize a One Health approach by integrating wildlife and domestic animal surveillance into outbreak investigations. Examples of PREDICT's contributions to disease outbreak response include:

Minimized Response Times for Outbreaks of Human Disease

Over the course of the project, dramatic improvements in response and control times were recognized. Unfortunately, the PREDICT team was not engaged in the West African region where the largest Ebola outbreak in history occurred during the end of PREDICT activities in 2014. Tragically, due to numerous causes, including lack of diagnostic capacity and public health education and infrastructure, the seriousness of the outbreak was not recognized until months after the first case occurred there. In contrast, in the Democratic Republic of Congo, where PREDICT was actively engaged with the government and the public health infrastructure,

samples were tested and control measures implemented within three days of collection of the first samples in a concurrent, yet separate Ebola outbreak that was then rapidly controlled before substantial spread. The rapid response and ultimately extremely reduced death toll and control time are illustrative of what can be achieved when a local One Health workforce is trained, employed, and able to activate during outbreaks.

Prevented Yellow Fever Outbreak in Bolivia

PREDICT rapidly identified a yellow fever outbreak in March of 2012 after five primates were found dead in eastern Bolivia. Laboratory diagnostics revealed that they were infected with a flavivirus. The Ministry of Public Health was immediately notified, and genetic sequencing performed by PREDICT confirmed that the infections had been caused by two yellow fever viral strains, both related to human cases occurring in Trinidad and Tobago and Brazil. The speed with which PREDICT was able to collect and test samples and interpret results (eight days) allowed local authorities to promptly implement human yellow fever prevention strategies (e.g. vaccination campaigns, public outreach, and mosquito control), resulting in zero human cases of illness during the outbreak.



PREDICT laboratory scientists, Placide Mbala and Ipos Ngay, preparing to process (inactive) samples from suspected Ebola virus disease cases in Boende, Democratic Republic of Congo.

A SUSTAINABLE MODEL FOR ENHANCED PREVENTION, DETECTION, AND RESPONSE TO VIRAL OUTBREAKS

USAID has recognized PREDICT's success in navigating the technical and management complexities of such a large-scale global initiative, citing the project's strength in its innovation and success in forging a shared vision and coordinated actions to protect public health. Through its One Health leadership and highly productive partnerships, PREDICT built a sustainable model for in-country and regional capacities to prevent, detect, and respond to viral outbreaks. PREDICT forged new networks of professionals from the human, animal, and environmental health sectors to promote global health through improving our understanding of high-risk interfaces for disease spillover from wildlife into people and implementing strategies for preventing and controlling emerging disease threats.

SURVEILLANCE STRATEGY



PREDICT's risk-based surveillance strategy grew from the need for a more proactive approach to pandemic prevention, in which pathogens are discovered early and at their source before they have the opportunity to emerge or spread widely in people. Surveillance efforts were focused on wildlife taxa most likely to serve as reservoirs for emerging zoonotic pathogens at human-animal interfaces with high-risk for disease transmission.

Conventional disease surveillance has traditionally relied on multiple, “siloe” data streams resulting from testing for expected pathogens, which can limit contributions to early detection of emerging zoonoses. PREDICT played a critical role in building capacity and linking a network of key stakeholders to develop a risk-based approach to disease surveillance. Partnerships with government ministries, scientific institutions, local organizations, and other key stakeholders allowed implementation of approaches that are integrated across the animal health, human health, and environmental sectors and standardized across all Emerging Pandemic Threat (EPT) priority countries.

An overall goal for enhancing surveillance in PREDICT countries was to explore the feasibility of sustainable, highly networked surveillance activities with the capacity to identify infectious disease threats of wildlife origin in regions most at risk of disease emergence. Proof of concept was demonstrated by the ability of participating countries to safely and effectively collect, transport, test, and report findings from significant numbers of samples collected from key locations and species of wild animals. Working on the ground with local teams, which included health professionals, scientists, educators, and ministry officials, PREDICT also identified areas and behaviors posing the highest risk for exposure to disease. We then detected and characterized viruses of unknown pandemic potential and identified likely animal reservoirs of zoonotic viruses with known epidemic potential at these high-risk interfaces to provide information needed to design intervention strategies that reduce human behaviors that facilitate disease emergence and amplification.

PREDICT's risk-based surveillance strategy was designed to identify viruses in situations where people have frequent, direct, and often intense contact with wild animals. In participating countries, a range of these high-risk disease transmission interfaces were targeted, often in areas with extensive anthropogenic impact to the landscape and vulnerable human populations, to discover novel potential pathogens where zoonotic diseases are most likely to emerge.

Wildlife being sold in a market in Northeastern Sulawesi, a priority area for PREDICT sampling.



PHOTO BY WILLIAM KARESH

HIGH-RISK WILDLIFE TAXA

Although many wild animal reservoirs of infectious pathogens remain to be identified, there are a number of demographic, ecologic, and behavioral characteristics (e.g. evolutionary and genetic relatedness to people, movement and population size, overlap among species, and social behavior) hypothesized to affect the likelihood of a wild animal serving as an important reservoir for zoonotic pathogens (Woolhouse and Gowtage-Sequeria 2005; Davies and Pedersen 2008; Luis et al. 2013; Smith and Wang 2013). To maximize discovery of zoonotic viruses with pandemic potential, PREDICT focused surveillance activities on wildlife taxa for which there was significant scientific evidence for high-risk pathogen transmission to humans (bats, nonhuman primates, and rodents) based on recent emerging and re-emerging viral zoonoses. To inform on surveillance activities, we identified animal hosts linked to spillover of all zoonotic viruses reported in the peer-review literature and, using network analyses, confirmed that the vast majority of zoonotic viruses recognized to date would have been detected by surveillance activities focused on bats, nonhuman primates, and rodents (Johnson et al. 2014).

Bats have been linked to several dangerous zoonotic pathogens including rabies, Ebola and Marburg filoviruses, Nipah and Hendra paramyxoviruses, Severe Acute Respiratory Syndrome (SARS)-like coronavirus (Calisher et al. 2006), and more recently Middle East Respiratory Syndrome (MERS)-like coronavirus (Memish et al. 2013). Bats are abundant and have several unique characteristics that make them well-suited to host, transmit, and spread viruses, including high species diversity; relatively long life span; capacity for long distance dispersal; and aggregation in dense, large, multi-species colonies (Calisher 2006; Turmelle and Olival 2009; Luis et al. 2013). Furthermore, many bat species are hunted and traded for food. In addition, bats live near humans and domestic animals, often roosting in houses or trees in villages and dense urban areas or in buildings and shelters on farms and food storage areas.

**Hunting practices
in a village in
central Lao PDR.**



PHOTO BY WCS LAO PDR

Rodents are reservoirs of several zoonotic viruses, including hantaviruses responsible for hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome, and arenaviruses causing lymphocytic choriomeningitis, Lassa fever, and various South American hemorrhagic fevers (Luis et al. 2013; Mills and Childs 1998). Many rodent species share environments with humans and livestock, frequently as a result of habitat encroachment for housing and agricultural intensification (Mills and Childs 1998). Rodents also serve as an important food source in many parts of the world.

Nonhuman primates also serve as sources of important pathogens for people, including Ebola virus, human immunodeficiency viruses, human T-lymphotropic virus, and simian foamy virus (Wolfe et al. 2004; Leroy et al. 2011; Sharp et al. 2011; Peeters and Delaporte 2012). Genetic relatedness between species increases the likelihood of cross-species transmission of pathogens (Davies and Pederson 2008; Parrish et al. 2008; Streicker et al. 2010), and nonhuman primates are close relatives of humans, thereby facilitating sharing of many zoonotic diseases. Primates are also infected with pathogens closely related to human pathogens (Wolfe 1998; Calvignac-Spencer et al. 2012). Contact between nonhuman primates and people is common, as primate species are frequently hunted and consumed in the tropics and inhabit many areas where human population growth is highest (Wolfe 1998; Calvignac-Spencer et al. 2012).

HIGH-RISK DISEASE TRANSMISSION INTERFACES

PREDICT targeted surveillance in geographic areas previously identified as emerging infectious disease hotspot regions (Jones et al. 2008), characterized by high wildlife biodiversity and intense anthropogenic pressures on shared resources. Local practices that foster intense human and animal contact are most likely to facilitate disease emergence, and PREDICT further targeted sampling of priority wildlife taxa at high-risk human-animal interfaces. Interfaces promoting transmission of zoonotic pathogens to people have occurred where diverse pools of wildlife pathogens come into direct and indirect contact with humans or their domestic animals, as was seen with SARS emerging from wildlife markets, Ebola and HIV/AIDS from butchering or handling of wild primates, and new strains of influenza in people handling poultry. High-risk interfaces reported in zoonotic disease transmission of viruses from wildlife to humans in the literature included contact with wildlife during hunting and in and around human dwellings and agricultural fields; occupational exposure, including veterinarians, researchers, and workers in laboratory settings; wildlife management; and zoos and sanctuaries (Johnson et al. 2014). These high-risk interfaces are important targets for pathogen surveillance and critical points for implementation of disease prevention and control measures.

To more fully investigate and rank the risk for pathogen transmission among important interfaces, PREDICT sampling activities targeted free-ranging wildlife in areas with varying land-use changes (e.g. deforestation, conversion to agriculture) at a number of high-risk disease transmission interfaces, including wildlife for sale in markets and restaurants; food value chain; wild animal farms; wildlife in sanctuaries, intensively managed protected areas, sites with ecotourism and in and around human dwellings and agricultural fields; wildlife preying on livestock, raiding crops, and causing public safety hazards; and hunted and consumed wildlife. Land-use changes were assessed in the context of how they could modify the risk of virus spillover by perturbing the dynamics of pathogens in wildlife hosts and/or by bringing novel host-pathogen pairs (including humans) into contact (see the Deep Forest section). Specifically, in Brazil, Uganda, and Malaysia, the Deep Forest study employed a standardized sampling procedure with a gradient design to evaluate the effects of land-use change and other key drivers on disease emergence risk (human contact, wildlife biodiversity, and viral biodiversity) and to test hypotheses generated through previous disease hotspots analyses (Jones et al. 2008).

WILDLIFE DISEASE SURVEILLANCE ACTIVITIES

Field teams of wildlife veterinarians experienced in the handling and sampling of live and dead wild animals led surveillance activities, ensuring humane and safe handling of animals and proper specimen processing and storage, while assisting in the development of a One Health workforce in wildlife disease surveillance. A variety of specimen types were collected and those types most likely to be involved with animal to human contact at the targeted high-risk interface were prioritized for testing. Because PREDICT sampled wildlife at high-risk interfaces using specimen types most closely aligned with disease transmission routes, viruses detected in wildlife samples collected during surveillance activities are among those with the highest rates of direct contact with human populations in hotspots for disease emergence. Data collected by field teams in PREDICT countries was matched with in-depth viral discovery efforts to identify animal hosts and high-risk interfaces most commonly associated with new and known viruses.

To overcome logistical challenges facing wildlife surveillance in remote regions, PREDICT also developed techniques that could enable sampling of free-ranging nonhuman primates (see Non-invasive Oral Sampling Technique box) when hands-on sampling was not feasible or permitted by law or religious traditions.

Developing Non-invasive Oral Sampling Techniques for Free-ranging Primates

Many high-risk human-nonhuman primate interfaces have not been monitored for viruses that could pose health risks to nearby human communities or conservation threats to endangered primates because of the difficulties inherent in hands-on sampling of primates. Several primate species are threatened or endangered and inhabit remote and logistically-difficult to access settings. To address this, PREDICT developed two novel sampling methods for detection of viruses in saliva – the distribution of chewable ropes and the collection of discarded forage food from primates (Smiley Evans et al. 2014).

Pilot studies to optimize and evaluate the effectiveness of the two techniques were performed. For the distributed rope technique, paired rope and oral mucosal swabs were collected from captive macaques at the California National Primate Research Center and tested for viruses. We were able to detect viruses in saliva samples collected from ropes distributed to captive macaques with the rope method performing similarly to the swab sampling method for detection of the target DNA virus, and with only slightly less sensitivity for the target RNA virus. Optimization of the discarded forage technique was performed with captive western lowland gorillas at the San Francisco Zoo. Field applicability of distributing ropes for sample collection was assessed with free-ranging nonhuman primates in Uganda, Rwanda, and Nepal. Viruses were also detected in discarded chewed plants from wild mountain gorillas and from free-ranging golden monkeys, indicating good virus recovery potential in dropped forage food (Smiley Evans et al. 2014).



These techniques have been applied more widely in PREDICT activities in other countries to enable sampling of nonhuman primates at interfaces that would otherwise not have been logistically feasible.

Rhesus macaque chewing on distributed rope at Pashupatinath temple, Kathmandu Nepal.

Site Characterization and Human-Wildlife Contact Surveys

Site characterization and preliminary human behavioral surveys were conducted at many of PREDICT's surveillance sites in order to better characterize the types of human-wildlife contact and the potential for transmission to humans for viruses detected in the targeted wildlife species sampled. Information collected at PREDICT field sites included general data on human and domestic animal (i.e. livestock) demographics; high-risk activities with wildlife in the area; direct and indirect contact with target wildlife species; and general ecology of bats, rodents, and nonhuman primates at the sites.

For the Deep Forest study (see Deep Forest section), which was implemented in Brazil, Malaysia, and Uganda, in-depth surveys were conducted to further characterize human-animal contact. Hundreds of households at each study site were surveyed to obtain descriptive data on frequency and type of contact with wild animals and domestic animals, such as livestock, along with livelihoods and household conditions relevant to zoonotic disease transmission. The survey was adapted and tailored to the country, sub-population, and setting in which it was implemented. The survey will be used to characterize risk of disease transmission at the landscape scale in each of three land-use gradients: disturbed, semi-disturbed, and pristine.

Syndromic Surveillance Activities

PREDICT also initiated surveillance activities based on triggers that signal a high potential for zoonotic pathogen outbreaks, including wild animal morbidity and mortality events with likely pathogen spillover into secondary hosts, and reports of zoonotic disease events in humans and domestic animals. Data reported in the literature have indicated that a surveillance strategy targeting healthy free-

ranging wildlife together with syndromic surveillance focused on reported mortalities in wildlife is ideal for virus discovery efforts (Levinson et al. 2013). Surveillance of apparently healthy wildlife will maximize zoonotic virus discovery potential, especially in bats and rodents, while syndromic surveillance, particularly focused on detection of disease outbreaks in nonhuman primate populations, will detect viruses most likely to impact human health.

To identify animal and human disease events warranting further investigation by PREDICT teams, daily summaries of disease outbreaks in humans and animals were reviewed using online sources of health event reporting, such as ProMed and HealthMap (see Information Management section). In addition, PREDICT launched a local media surveillance (LMS) pilot study in seven countries to monitor health events in local media sources and assess the value and utility of LMS to enhance surveillance and improve early recognition of disease events (see LMS box and Information Management section). Furthermore, an animal mortality monitoring program (AMMP) and field pathology program (FPP) were developed in Uganda to obtain reports of animal die-offs and assess their utility for detecting and determining causes of mortality in the events. As reports were received, PREDICT assessed the need for enhanced surveillance and outbreak investigation and worked alongside government and international authorities in responding to outbreak situations (see AMMP and FPP box).

Local Media Surveillance (LMS) Study

PREDICT piloted a LMS study in seven countries (Bangladesh, Bolivia, Cambodia, Cameroon, Nepal, Tanzania, and Uganda) to evaluate the potential for LMS to enhance global digital disease surveillance (Schwind et al. 2014). This study demonstrated that monitoring health events reported in local print media can be an effective and worthwhile addition to active digital surveillance networks in regions with less-developed capacity for disease detection and response.

For this pilot study, sources were chosen from a media inventory gathered by local PREDICT staff. Approximately 3-6 relevant sources (e.g. local language newspapers) not already feeding into main aggregation sites, such as Google News or HealthMap, were scanned each week for health event-related articles by trained in-country personnel, who were often able to conduct the monitoring during normal work down time. Reports were sent to HealthMap for inclusion in their digital surveillance system and then compared to global reports to identify unique and overlapping health event coverage.

Local media surveillance filled gaps in global digital surveillance network coverage by contributing valuable localized information on disease events. Approximately 67% of all submitted LMS reports were not otherwise captured by HealthMap (Schwind et al. 2014). Local media surveillance also contributed unique and useful information on local disease events. It was easy to implement and required minimal resource commitments. Furthermore, unlike digital media surveillance programs, LMS supported diverse languages.

**Bwindi Community
Hospital Buhoma,
Uganda.**

HUMAN DISEASE SURVEILLANCE

Four pilot study efforts were employed to further assess human risk at high-risk interfaces and detect virus spillover events in these populations: 1) an in-depth behavioral and prospective biological sampling effort in partnership with a rural community hospital in Uganda; 2) a cohort study in high-risk populations at human-animal interfaces in Southern China; 3) a disease of unknown origin (DUO) study for highly pathogenic human infections in Central Africa and Southern China; and 4) a retrospective analysis of repositories of acute human specimens in high risk regions (Deep Freeze study).



PHOTO BY TIERRA SMILEY EVANS

Bwindi Zoonoses Study in Southwest Uganda

PREDICT piloted an in-depth behavioral and prospective biological sampling effort in partnership with the Bwindi Community Hospital in order to better understand exposure to zoonotic viruses from wildlife in humans and to identify communities whose activities place them at risk. The Bwindi region is a remote area of southwest Uganda predominantly inhabited by Batwa and Bakiga tribes, their domestic livestock, and a large diversity of wildlife. The Batwa traditionally lived in the Bwindi Impenetrable National Forest as hunter-gatherers. Bakiga are mainly agriculturalists and have close contact with domestic

animals. Patients presenting to the Bwindi Community Hospital and Byumba Health Center II with symptoms suggestive of viral infection were enrolled in this pilot study. Blood samples and oral swabs were collected from each patient. In addition, a medical and behavioral questionnaire was administered to volunteers. Using PREDICT viral family PCR protocols, a number of viruses have already been detected and are undergoing further characterization. As active shedding of many viruses is rare, exposure to zoonotic viruses will also be evaluated by serology. A number of potential risk factors for exposure to zoonotic viruses have been identified from behavioral questionnaire data (i.e. hunting and consumption of wildlife species), and risk factor analyses will identify activities that are directly increasing risk for contracting zoonotic viruses.

Cohort Study

The purpose of the cohort study was to assess the presence of zoonoses of pandemic potential in humans with high exposure to wildlife and associated products. Twelve rural cities were chosen in Guangdong Province as sites with human populations with high-risk human behaviors (i.e. elevated exposure to wild animal blood or bodily fluids through butchering or hunting). Serum samples were taken from consenting participants who also responded to a detailed questionnaire that focused on demographics, work practices, perceptions, and potential exposures to zoonotic infections. PREDICT followed up with individuals who had serologic evidence of exposure to hantavirus, SARS Coronavirus, and Severe Fever with Thrombocytopenia Syndrome (SFTS) virus (an emerging, potentially tick-borne virus recently discovered in China), asking them to participate in an additional sampling visit and to invite up to three of their close contacts to enroll in a follow-up study. PREDICT collected additional samples and behavioral data in order to investigate transmission through close contact and gauge transmissibility of the viruses. In the questionnaire, almost all participants (94%) indicated that they had touched live or dead wild animals. In addition, butchering (81%) and eating (72%) wild animals were the most common types of animal interactions, and injuries related to working with wild animals was reported by 23% of participants.

Animal Mortality Monitoring Program (AMMP) and Field Pathology Program (FPP) in Uganda

PREDICT piloted a novel cellular phone-based animal mortality monitoring program as an early warning system for outbreaks of infectious diseases in Queen Elizabeth Conservation Area, Uganda. Because noticeable sickness or die-offs in animals can serve as an alert system for zoonotic diseases, monitoring of sick and dead animals potentially provides a means to detect emerging zoonotic pathogens before they spillover into the human population.

During their daily patrol, wildlife rangers alerted authorities to wildlife morbidity and mortality events using mobile phone technology. Over 1,586 records of sick ($n = 741$) and dead ($n = 845$) animals from greater than 30 species were reported using this system. Among these reports, 135 cases were identified by rangers as potentially disease-related.

In conjunction with the animal mortality monitoring program, the field pathology program (FPP) responded to 10 field reports of wildlife mortalities in the spring of 2014. Through the FPP, PREDICT provided supplies, training, and support to the Pathology Department at Makerere University and enabled sustained pathology training opportunities through the Smithsonian Institute. Causes of death included infectious disease, trauma, and suspected toxicity. All of the mortalities were close to or within human inhabited areas, and several of the carcasses were butchered and consumed by people in local communities highlighting the potential for transmission of zoonotic pathogens through shared environment or through direct contact and consumption.

Although not all cases were the result of zoonotic pathogens, the program illustrated that 1) animal mortality events can be reported in real-time; 2) the methodology is a simple, fast, effective, easily replicable, and inexpensive means to report morbidity and mortality; 3) sample retrieval for laboratory diagnostics is feasible; and 4) when paired with a pathology program, it can be an effective means to document and monitor causes of mortality and potential pathogenicity of detected infectious agents in animal hosts.

Among participants who reported butchering wild animals, 92% indicated that they used a machete, and 64% indicated that they used their bare hands for the butchering process. In addition, 25% of respondents reported butchering-related injuries.

Diseases of Unknown Origin (DUO) Study in Central Africa and Southern China

The study of DUO provides an important tool for the identification of novel emerging infectious diseases. In Central Africa, PREDICT examined samples from people collected during a series of small outbreaks of suspected viral hemorrhagic fever that occurred from 2003 to 2014 that had negative results when examined with standard clinical diagnostic tests. Ebola or Marburg viruses were first suspected in each outbreak; however, subsequent laboratory analysis ruled out infection with known filoviruses or other usual causes of viral hemorrhagic fever. Virus family level and virus-specific RT-PCR testing for known hemorrhagic fever viruses was initially conducted, and samples for which an etiologic agent was not detected were shipped to the US-based PREDICT partner laboratory of Dr. Charles Chiu at the

University of California, San Francisco, for analysis by deep sequencing (“deep” sequencing is an un-biased strategy for detection of novel infectious agents – the major advantage of deep sequencing is the capacity to detect pathogens at low levels or that are genetically divergent from known microorganisms).

In addition to detecting over 20 previously identified viruses, this study led to the discovery of a novel rhabdovirus (Bas Congo virus; BASV) associated with cases of acute hemorrhagic fever in DRC. This is the first time that this viral family has been linked to this syndrome, putatively increasing the number of viral families that cause viral hemorrhagic fever to five. A serological test was rapidly developed in order to support the characterization of this new virus and allow for expanded investigation of the epidemiology. The newly developed assay serves as a proof of concept that serological tests can be quickly created once a novel zoonotic virus is discovered to facilitate a more thorough evaluation of the level and extent of the risk it poses to public health.

In Southern China, PREDICT established a hospital-based syndromic surveillance study in Guangdong Province to increase capacity for the identification of new and previously undetected pathogens. Long-term collaborative relationships were established with 16 sentinel hospitals in Guangdong Province through the Centers for Disease Control of Guangdong Province (GDCDC) and the Guangdong Institute of Public Health (GDIPH). In participating sentinel hospitals, samples from patients exhibiting symptoms of encephalitis, hemorrhagic fever, fever with thrombocytopenia, fever with rash and/or diarrhea, and influenza-like illness that tested negative for normative diagnostics were sent to GDCDC for additional analysis using PREDICT protocols. These samples were tested for a variety of viral families based on symptoms. These viral families included but are not limited to seadornavirus, flavivirus, paramyxovirus, hantavirus, arenavirus, hantavirus, and filovirus using PREDICT protocols at GDCDC.

Deep Freeze

The goal of the Deep Freeze study was to evaluate the utility of the PREDICT viral screening procedures for further screening of archived clinical samples from humans for which specific pathogens have already been excluded based on laboratory testing. The study was designed to evaluate the utility of PREDICT diagnostic laboratory protocols for detecting viruses in archived human samples that had been stored in a variety of conditions and for variable lengths of time, and therefore with variable likelihood of viral detectability, and make recommendations for how samples can be archived in the future to maximize virus discovery. PREDICT polled partners in PREDICT countries for availability of specimens that 1) were derived from human populations with high contact rates with wild animals or products, and 2) were acquired from individuals with clinical symptoms of an infectious disease (e.g. fever and encephalitis). Highest priority was given to samples from longitudinal collections, where the most-likely cause(s) of disease (e.g. malaria, dengue, influenza) have already been ruled out based on laboratory testing, and for which supporting data was available (e.g. geographic location, date sample was collected, type of sample, clinical presentation and outcome, and known risk factors).

Six PREDICT countries had specimens meeting criteria for inclusion in the study: Cameroon, DRC, Gabon, China, Indonesia, and Malaysia. Specimens from these collections were derived from individuals presenting with acute febrile, encephalitis, respiratory, and hemorrhagic syndromes. Samples were obtained while patients were symptomatic. A total of 1,415 archived specimens from acute human patients were analyzed using PREDICT diagnostic laboratory protocols, followed by on-going genetic sequencing for positive samples. As expected, archived

specimens for which the specimen type was the most appropriate to test based on the clinical syndrome (e.g. oropharyngeal swabs with respiratory illnesses) had the highest virus detection prevalence. In addition, detection was highest for samples maintained at ultralow temperatures.

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VIRUS DETECTION AND DISCOVERY



PREDICT's strategy for virus detection and discovery evolved from the need for a cost-efficient approach that produces specific, high-resolution data and allows for detection of known and new potential pathogens in tandem. It was critical that it could be effectively implemented in countries with the most limited resources around the world. The PREDICT team, therefore, had to rapidly and creatively adjust to produce the results needed for all aims of the project.

Over just five years, PREDICT developed and optimized protocols for the detection and discovery of viruses from target viral families that had been recognized in animal hosts and caused disease and epidemics in people, enhancing laboratory capacities in 20 countries and resulting in the detection of 984 unique viruses in animals and humans.

VIRAL DISCOVERY AND DETECTION HIGHLIGHTS

In animals, PREDICT identified 812 novel viruses and 147 known viruses (only 15% of total detected viruses were discovered before the PREDICT project began or by teams not involved in the PREDICT project; see Global Achievements section). Pilot work on human disease surveillance conducted in Uganda, the Democratic Republic of Congo, Cameroon, Gabon, Malaysia, China, and Indonesia yielded 34 viruses (3 novel and 31 known; some of the known viruses also being found in animal samples). Both RNA and DNA viruses were targeted for testing, and known and new viruses were detected in 28 viral families or genera (Table 1). This is the most comprehensive and systematic viral discovery effort to date and includes the detection of many significant human and animal pathogens such as Sudden Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) related coronaviruses, and the discovery of many novel viruses closely related to pathogens of zoonotic concern. By the end of 2009 and the year the PREDICT project began, 590 mammal viruses had been recognized by the International Committee on Taxonomy of Viruses (ICTV). Therefore, not only was PREDICT able to detect known viruses, some of which are pathogenic in people, but we detected more novel viruses than those already previously recognized by the ICTV. This detection and discovery success demonstrates the significant utility of the PREDICT strategy in global surveillance and provides data that can contribute directly to better pandemic preparedness.



PHOTO BY GORILLA DOCTORS

Table 1. Number of known and new viruses by viral family in each wildlife host taxa*.

Viral family	Novel Bat	Known Bat	Novel Primate	Known Primate	Novel Rodent/Shrew	Known Rodent/Shrew	Novel Human	Known Human
Adenovirus	53	3	6	4	32	1	1	3
Astrovirus	153	33	19	3	31	1	0	1
Coronavirus	61	30	3	0	6	0	0	2
Dependovirus	0	0	11	0	0	0	0	0
Flavivirus	3	0	0	1	0	0	0	2
Hantavirus	3	1	0	0	0	2	0	1
Herpesvirus	46	0	48	25	43	6	0	5
Orbivirus	1	0	1	0	0	0	0	0
Paramyxovirus	63	7	0	2	11	2	0	3
Polyomavirus	27	1	4	3	8	0	0	1
Arenavirus	0	0	0	0	2	2	0	0
Rhabdovirus	19	0	2	0	7	0	1	0
Seadornavirus	1	0	0	0	0	0	0	0
Bocavirus	0	2	1	3	0	0	0	0
Enterovirus	0	0	5	4	2	0	0	5
Retrovirus	0	0	4	7	0	0	0	1
Alphavirus	0	0	0	1	0	0	0	0
Poxvirus	0	0	0	1	1	0	0	0
Influenza	0	2	0	0	0	1	0	5
Mononegavirales	0	0	1	0	0	0	0	0
Papillomavirus	0	0	1	0	0	0	0	0
Picobirnavirus	0	0	120	0	0	0	0	0
Picornavirus	0	0	4	0	0	0	0	0
Picornavirales	0	0	4	0	0	0	0	0
Phlebovirus	1	0	0	0	0	0	0	0
Rotavirus	0	0	1	0	0	0	0	0
Anellovirus	0	0	0	0	0	0	1	1
Hepadnavirus	0	0	0	0	0	0	0	1

*Note numbers of viruses do not total to 984 as viruses have been found in more than one wildlife host taxa.

Coronaviruses in Bats

The emergence of two beta coronaviruses, SARS-CoV and MERS-CoV, as the causes of fatal respiratory disease in people emphasizes the importance of surveillance for coronaviruses that have potential to spillover from bats into the human population. To date (i.e. 2014), PREDICT has detected almost twice the number of new coronaviruses than were previously acknowledged species (both approved and proposed) by the ICTV by the end of 2009. As part of this effort, PREDICT identified a number of coronaviruses in bats. Examples include:

Coronaviruses in Bats in Mexico. In a screen of 606 bats from 42 unique bat species in Mexico, we identified 13 distinct coronaviruses (12 of which were novel; Anthony et al. 2013b). Analyses of these viruses in the context of their hosts and ecological habitat indicated that the host species is a strong selective driver in coronavirus evolution and that a single species/genus of bat can harbor multiple coronaviruses. Significantly, a beta coronavirus with 96.5% amino acid identity to MERS-CoV was found in a broad-eared bat (*Nyctinomops laticaudatus*), suggesting that MERS may have origins in bats (Anthony et al. 2013b). Studies like these in PREDICT are important to our understanding of how viral diversity relates to host diversity and of how viruses cross host species boundaries to emerge in people.

Coronaviruses in Bats in China. PREDICT investigators also isolated for the first time and characterized the binding receptor from a SARS-like coronavirus from a Chinese horseshoe bat (*Rhinolophus sinicus*; Ge et al. 2013). Results showed the virus was able to bind to the human ACE-2 cell receptor suggesting that direct transmission to humans from bats is possible. Previously, the only known source of SARS for humans was civets sold in markets. During the 2003 outbreak of SARS in the wet markets of Guangdong province in China, it was thought that bat viruses first infected civets and then evolved to infect people through this intermediate host. However, this study provides compelling evidence that an intermediate host was not necessary (Ge et al. 2013). Isolation of the live SARS-like virus from bats will allow for future studies to identify potential effective control measures, including vaccine development.

DIAGNOSTIC APPROACH

Virus detection across high-risk interfaces for PREDICT was performed using a combination of consensus polymerase chain reaction (cPCR) and high through-put sequencing (HTS). This strategic approach combines high sensitivity with broad reactivity (i.e. detects viruses at low levels while casting a wide net) and allows the detection of both known and novel viruses in a wide range of samples and host species (Goldstein et al. 2013; Anthony et al. 2013 a,b). cPCR was the primary method used. This approach permits the “universal” amplification of viruses within a given viral family or genus, and the subsequent discernment of viral strains. Over the past five years, PREDICT has developed and optimized detection protocols for numerous viral families of concern, including: alphaviruses, arenaviruses, astroviruses, bunyaviruses (both orthobunya- and hantaviruses), coronaviruses, filoviruses, flaviviruses (both blood-borne and insect-transmitted), herpesviruses, orthomyxoviruses, paramyxoviruses, poxviruses, reoviruses, retroviruses, and rhabdoviruses. While cPCR has been used for decades in research laboratories, it was PREDICT that recognized the usefulness of this method for global surveillance, diagnostics, and virus discovery initiatives (it is inexpensive and easy to implement in resource-constrained countries) and invested in the development of this strategy. This strategy included the design and construction of two synthetic ‘universal controls’ which provided all laboratories with access to much-needed and standardized control material without any danger of pathogen transmission. HTS was used as a secondary (complimentary) method to the cPCR viral detection and discovery. It is far more expensive than cPCR and requires teams of diagnosticians and bioinformaticians that are generally only available in sophisticated laboratories. HTS is, however, currently the most advanced method available for viral discovery, as it works by generating sequence data for all nucleic acids present in a given sample. For this reason, HTS was used to complement the cPCR testing and applied to a strategic subset of samples. The rationale was to use HTS to capture any viral diversity missed by consensus PCR; for example, viruses that are divergent or not among the initial targeted viral families and also, thereby, allowing for refinement and validation of the cPCR assays over time.

A significant benefit of the PREDICT strategy is that it extends beyond the detection of viruses in wildlife and can be successfully applied in other areas – such as the diagnosis of mystery illnesses in medical hospitals and veterinary laboratories where testing capacity has historically been quite limited, but basic equipment such as thermal cyclers for conventional PCR may be available. As such, the PREDICT approach has had far reaching implications for the implementation of current global surveillance and virus detection and discovery efforts. An additional benefit of the PREDICT strategy is that microbes in the samples are killed during nucleic acid extraction, which allows work to take place in countries that do not have sophisticated lab biosecurity without increasing exposure to lab workers to dangerous pathogens.

DATA MANAGEMENT AND REPORTING

Once viral sequences were identified by either cPCR or HTS, the data were uploaded by personnel in each country into the secure surveillance database (see Information Management section). On a monthly basis, all sequences generated globally for PREDICT were then reviewed in order to provide interpretations of the data back to host governments (for approval to release the data publicly) and to summarize the viruses detected. Viruses were deemed ‘novel’ if genetically divergent from known strains/species and classified as ‘known’ if they were sufficiently similar to those previously reported in the public domain. These genetic ‘cut-offs’ were determined by assessing the genetic distance between viral species already ratified (or about to be) by the International Committee for the Taxonomy of Viruses (ICTV). Each viral family, therefore, had a different “cut-off” that was used to assess novelty. Where known viruses were detected, PREDICT has provided interpretations of whether that particular virus is a known pathogen of animals or people. Where novel, PREDICT has provided interpretations of the potential risk that virus poses – though in most cases this was not possible to do with any real certainty due to the pure uniqueness and limited sequence available of the detected viruses. Instead, PREDICT provided comment on potential risk factors that might be useful for selecting new viruses for further

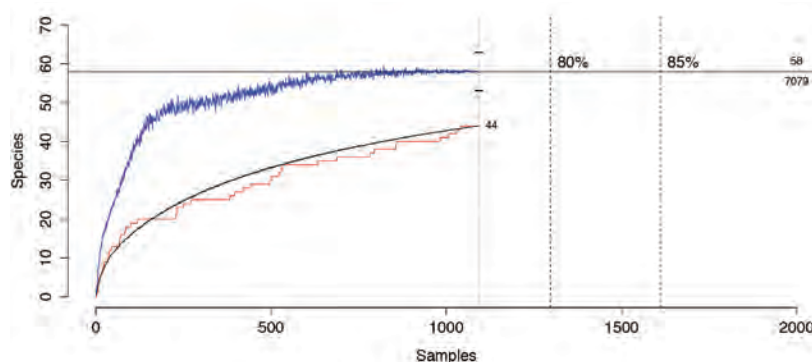


Figure 1. Number of samples versus the number of new species of viruses. The actual number of new viruses found in each sample is shown in red. The number of new viruses discovered in these samples is 44. The estimated discovery curve is shown in black. The blue line is the Chao2 estimator, which is used to calculate the maximum number of new viruses. In this case, we expect to find 58 new species of viruses. In order to identify 100% of the new viruses, a total of 7,079 samples would need to be tested. However, if we aim to identify 80% of the new species of viruses, only a little more than 1,300 samples would be needed. Based on this methodology, we will be able to develop optimal surveillance strategies and sample size targets.

Adapted from Anthony et al. 2013a.

characterization for assessing spillover, amplification, and spread risk, such as number of hosts in which it was found, its relationship to a known pathogen, or the identification of that virus at a high-risk transmission interface. Once cleared for release by host country governments, data were shared on the PREDICT public site (www.healthmap.org/predict) in a user friendly format for stakeholders, the public health community, and the general public.

VIRUS DISCOVERY CURVES

PREDICT provided the first ever robust estimate of total diversity of known and novel viruses in a mammalian species using data generated from screening 1,093 samples (urine and throat swab) collected from *Pteropus giganteus* fruit bats from Bangladesh (Anthony et al. 2013a). The specimens were screened for nine viral families: paramyxoviruses, coronaviruses, adenoviruses, astroviruses, herpesviruses, polyomaviruses, bocaviruses, hantaviruses, and influenza A, using PREDICT viral detection and discovery protocols. Viral discovery curves were generated by applying established ecological (statistical) approaches in order to estimate the number of undiscovered viruses that exist in a given population, as well as the number of samples required to optimize surveillance (Figure 1). Such analyses are important because they demonstrate that the size of the ‘zoonotic pool’ may not be as large as often believed, that the cost of global surveillance and discovery efforts could well be far less than the cost of a single pandemic, and that PREDICT could (over time) make a significant contribution to our knowledge of viral diversity in wildlife.

VIRUS DETECTION BY SPECIMEN TYPE

Because the goal of the PREDICT project was to detect new viruses with the potential for causing illness in humans, it was most important to pick the best sample, that which reflects the contact and thus potential route for exposure to people, for testing. Specimen type selection was based on the interface and the potential route of transmission of the virus, so thinking about how the humans were coming in contact with the wild animals and therefore potential pathogens that the animals may be carrying. For example, if exposure was thought to occur through animal bites, then saliva was tested, versus if exposure was thought to occur through aerosolization of virus due to cleaning up animal feces, then feces were tested. Thus, viral detection and discovery was maximized by live animal sampling targeted according to specimen type in conjunction with the type of contact at the likely transmission interface. As part of the PREDICT project, we also tracked which specimen types were best for detecting known and new viruses in each viral family to evaluate if our strategy needed to be revised (Figure 2). Results to date support our testing of feces and swab samples to detect viruses that may be transmitted from animals to people.

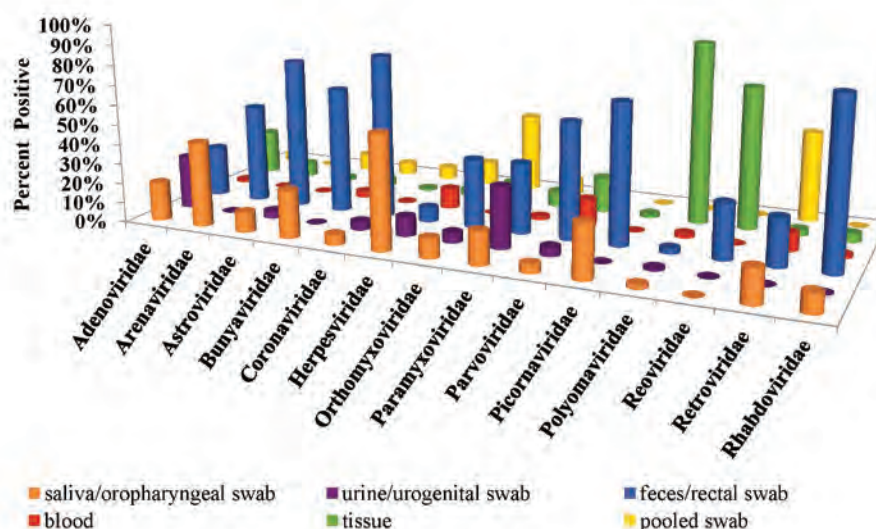


Figure 2. Proportion of each specimen type testing positive by cPCR for each viral family or genus tested by the PREDICT project.

LABORATORY CAPACITY BUILDING

A key focus in the first year of the PREDICT project was to better understand the baseline capacities for conducting surveillance and performing laboratory testing of samples collected from wildlife to detect viruses in hotspots regions where viral pathogens are likely to emerge. As part of this effort, in the second year, we visited many countries and laboratories to better understand in-country and regional diagnostic laboratory capacities for viral detection. Over time, laboratory partners were identified in each country and included national, inter-governmental, university, and private laboratories.

PREDICT first began working with laboratory partners to identify needed improvements in infrastructure and safety procedures. Over time, laboratory protocols and control materials were provided along with training, guidance, and support to implement, perform, and optimize virus detection protocols. A large emphasis was also placed on training for sample and test result tracking, data management, and results sharing. Where possible, linkages were made among laboratories and countries so that regional support became available for diagnostics and training.

The implementation of PREDICT laboratory protocols as a core capacity was tracked through the course of the project (see Capacity Building section). As a result of PREDICT's efforts to build laboratory capacity to detect known and new viruses, 27 laboratories were screening for target viral families by the end of the project compared to that capacity being available in six laboratories at the onset when baseline capacity was assessed. Further, four additional laboratories have received training in preparation to begin to screen for viral families in the last year of PREDICT activities (Figure 3 and Table 2), and among those PREDICT partner laboratories not performing their own screening for target viral families, all had access to diagnostic capacity through regional or global laboratory networks.

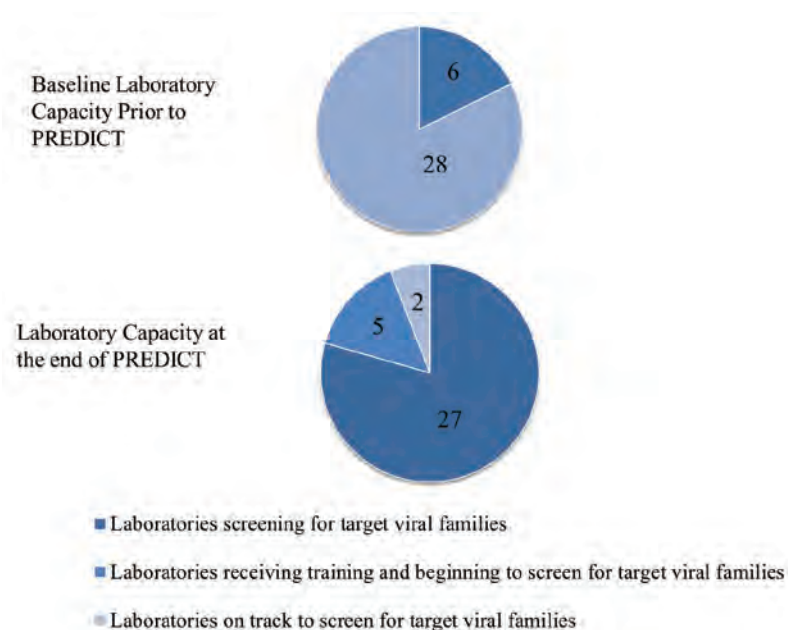


Figure 3. Comparison of diagnostic laboratory capacities for screening for target viral families among partner laboratories within PREDICT countries at the onset and end of the first phase of the PREDICT project (2009-2014).

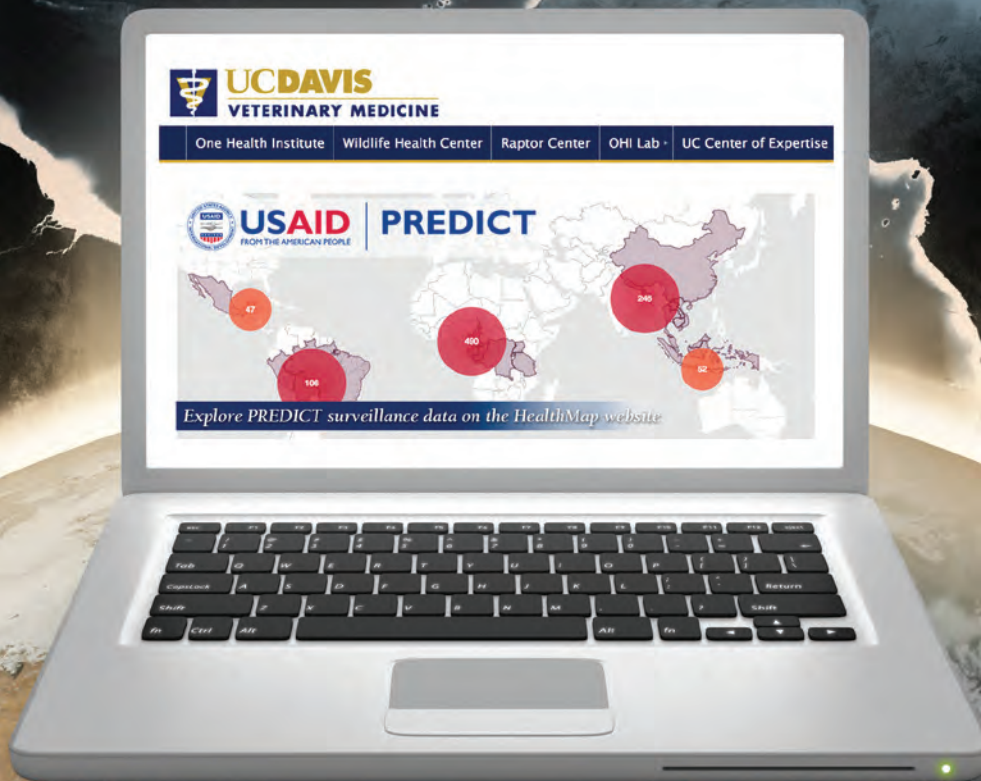
Table 2. Number of collaborating laboratories by region that were targeted for training and implementation of viral family PCR protocols.

Region	Number of labs targeted for screening with desired viral families	Number of labs receiving training in preparation for screening of desired viral families	Number of labs that have initiated work that will lead to screening desired viral families (labs with full or partial capacity)
Africa	7	7	5
Asia / SE Asia	19	19	16
Latin America	8	6	6
Totals	34	32	27

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INFORMATION MANAGEMENT



Access to accurate, comprehensive, and timely surveillance data is critical for predicting and responding to emerging diseases of wildlife origin. Information must be readily accessible and flow seamlessly across institutional, disciplinary, and international boundaries. Likewise, archived biological samples must be properly maintained, easily identified, and readily available for further analyses in order to confirm a pathogen's role in an emerging disease outbreak.

PREDICT developed a system comprised of a secure internal data collection and management wildlife surveillance database linked to an open access, online platform integrating field and digital surveillance data with epidemiological risk modeling in order to facilitate prevention of and timely detection and response to emerging zoonotic diseases. Human surveillance data was managed using a different system due to ethical requirements. For ease of conceptualization, information flow is displayed below in three compartments (Figure 1); however, in reality information flow was dynamic, and the boundaries among these categories were fluid.

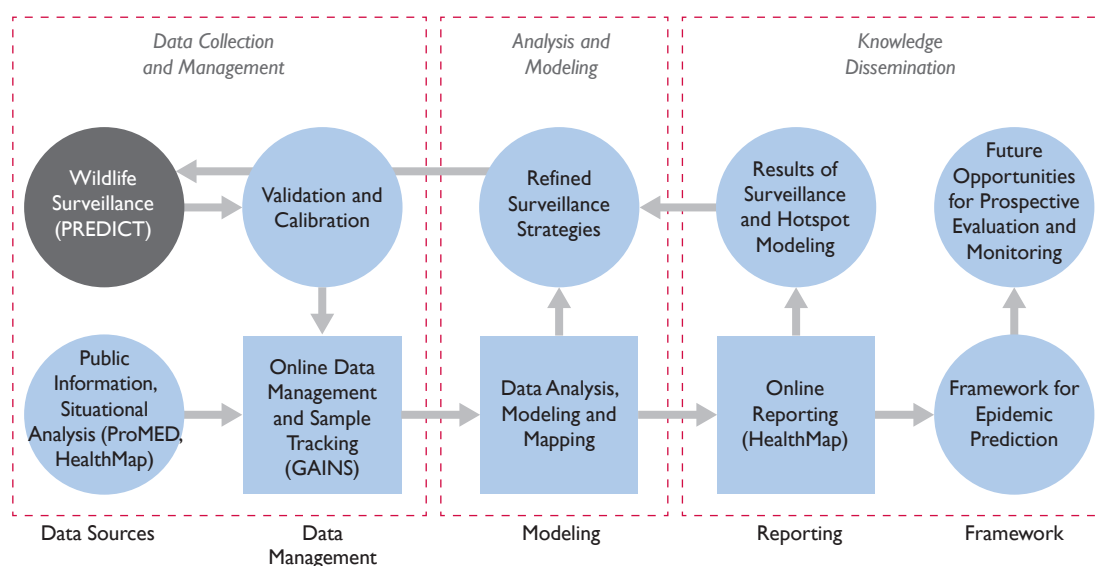


Figure 1. Overview of wildlife disease surveillance information management in PREDICT.

Compartment #1 – Data Collection and Management

Collection and integration of standardized data from wildlife disease surveillance and public information sources was a key goal of this project. PREDICT developed protocols and conducted training to ensure that standardized surveillance data was collected and recorded by all partners. Wildlife field surveillance data were integrated and managed within the internal, secure PREDICT database Global Animal Information System (GAINS) system. Originally developed with funding from USAID and Centers for Disease Control and Prevention (CDC) to track

avian influenza in wild birds, GAINS is a collection of open- and closed-source applications that provide mechanisms for input, management, and visualization of data. Locally appropriate policies on data ownership, governance, and release were developed in collaboration with host-country officials, partners, and USAID. PREDICT used multiple approaches for data quality control and validation and provided feedback reports to project partners (Validation and Calibration stage; Figure 1). Because field surveillance data alone may have been insufficient to detect novel events, publicly available information (i.e. digital surveillance data collected by ProMED and HealthMap) on emerging diseases was also collected, geo-referenced, and integrated with surveillance data.

Compartment #2 – Analysis and Modeling

The synthesized digital and field surveillance data were collated, analyzed, and used in computational models to guide targeted surveillance and to develop methods to predict disease emergence. Epidemiologists monitored the digital and field surveillance data on a real-time basis, in cooperation with field staff with local knowledge and expertise, to identify trends in virus detection and distribution, as well as in emerging disease events to provide ongoing status reports to stakeholders (see below).

Compartment #3 – Knowledge Dissemination

The principle means of distribution of field and surveillance data and test results is through a public data sharing and visualization platform hosted by the HealthMap project (www.HealthMap.org/predict). This platform provides interested individuals and organizations around the world access to detailed information obtained from a variety of data feeds on both known and emerging zoonotic diseases in hotspot areas worldwide (see below and Table 1). HealthMap.org/predict has a wildlife-specific data stream, which collects data from wildlife surveillance sources (e.g. World Organization for Animal Health (OIE), Wildlife Data Integration Network

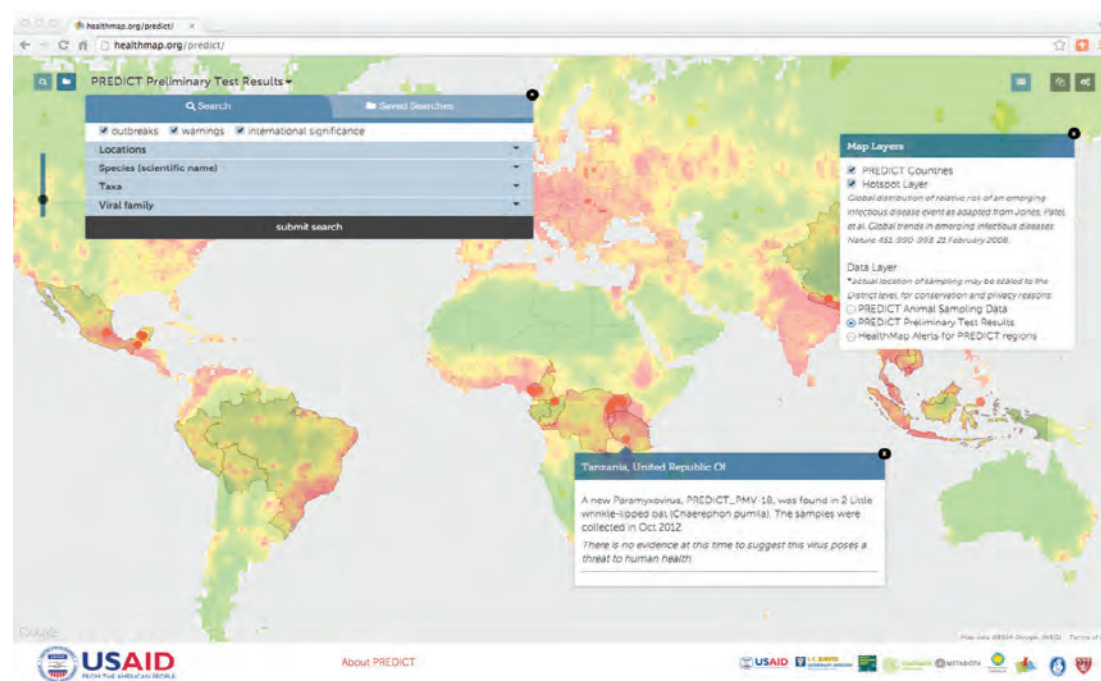


Figure 2. Screenshot of the public facing PREDICT portal (healthmap.org/predict).

(WDIN)), as well as from publicly available information sources on emerging diseases (e.g. ProMED-mail; Figure 2). Layered on top of these digital surveillance data are the field surveillance data collected by PREDICT on wildlife disease outbreaks and standardized surveillance and test result data, as well as the results of epidemiological hotspot modeling conducted by the PREDICT modeling team.

Interpreting and sharing of information with the host governments in PREDICT countries involved steps in the process of data collection, management, validation, and verification that are often overlooked. All data were examined at entry and later upon integration into the system, for completeness, accuracy, and logical consistency. Once all test results (e.g. initial detection and subsequent sequencing of viruses) were available for a given specimen, the results were interpreted in light of all available scientific literature by PREDICT virologists. This iterative process ensured the highest quality, most robust, data possible. After this process of examination and interpretation was complete, data were provided to host governments for examination, used to inform policy, and approved for public release on HealthMap. PREDICT project activities, results, and products, including protocols and guides, were highlighted and made publically available through the University of California, Davis PREDICT website (<http://www.predict.global>).

HealthMap

Digital surveillance of web-based data sources facilitates early outbreak detection and also supports increasing public awareness of disease outbreaks prior to their formal recognition. Web-based news media provide an alternative public health information source, particularly in resource-poor areas.

Table 1. Comparison of Healthmap, the PREDICT disease event-based reporting system, with other event-based systems (Adapted from Keller et al. 2009).

Table. Characteristics of 3 primary systems that process event-based informal data sources*						
System	Data source (languages)	Data characterization	Information formatting	Data dissemination		
				Access	User interface	Format
GPHIN	Factiva, Al Bawaba (9 languages)	Automatic and human	Categorization, machine translation, geocoded	Subscription only	Boolean and metadata query system (native)	Email alert
HealthMap	Google News, Moreover, ProMED, WHO, EuroSurveillance (4 languages)	Automatic	Categorization, geocoded, time coded, extra information	Open	Mapping, faceted, browsing (native)	RSS feed
EpiSPIDER	ProMed, GDACS, CIA Factbook (English only)	Automatic	Categorization, geocoded, time coded, extra information	Open	Web exhibits, faceted browsing (imported)	RSS, JSON, KML feeds

*GPHIN, Global Public Health Intelligence Network; WHO, World Health Organization; RSS, Really Simple Syndication; EpiSPIDER, Semantic Processing and Integration of Distributed Electronic Resource for Epidemics (and disasters); GDACS, Global Disaster Alert Coordinating System; CIA, Central Intelligence Agency; JSON, JavaScript object notation, KML, keyhole markup language.

HealthMap, founded in 2006, is an established global leader in utilizing online informal sources for disease outbreak monitoring, with over a million users a year. The freely available Web site, available at healthmap.org, delivers real-time intelligence on a broad range of emerging infectious diseases for a diverse audience including libraries, local health departments, governments, and international travelers. HealthMap brings together disparate data sources to achieve a unified and comprehensive view of the current global state of infectious diseases and their effect on human and animal health (Table 1). HealthMap relies on a variety of electronic media sources, including online news aggregators, eyewitness reports, expert-curated discussions, and validated official reports. Through an automated process, updating 24/7/365, the system monitors, organizes, integrates, filters, visualizes, and disseminates online information about emerging diseases in nine languages, facilitating early detection of global public health threats.

Additional Applications and Further Explorations of Information Management Tools

Novel Digital Surveillance Tool for Monitoring the Wildlife Trade. Through the HealthMap platform, PREDICT and partners contributed to an open source automated web crawling surveillance method designed to monitor official and unofficial reports of illegally traded wildlife and wildlife products (Sonricker Hansen et al. 2012). The trade in wildlife and wildlife parts has been identified as a potential avenue for novel diseases to enter human populations, as the SARS and monkeypox outbreaks have demonstrated. Unfortunately, due to its clandestine nature, no comprehensive database exists on the scope, scale, and extent of the wildlife trade. Building on tools developed for early disease detection using the internet, PREDICT and HealthMap created a site that collects information from the global digital media on wildlife trade that allows at least an index of the wildlife trade network. Available at <http://www.healthmap.org/wildlifetrade>, the digital wildlife trade surveillance tool is open-source and presents illegal wildlife trade reports worldwide in near real-time using an interactive visualization display (Figure 3). The system collects official and unofficial reports based on keyword searches and classifies reports on the



Figure 3. HealthMap wildlife trade surveillance output (healthmap.org/wildlifetrade) showing illegal wildlife trade reports received through the automated system during 2014 (sourced on August 19, 2014).

illegal wildlife trade by location and species prior to overlaying the information onto the map. The resulting system continually aggregates and displays the data to provide insight into the global wildlife trade network.

HealthMap's PathFinder Decision Support Tool. In collaboration with the Emerging Pandemic Threats Extractive Industries Working Group, PREDICT initiated the development of a series of decision support tools designed to supplement existing health impact assessments and to provide an application for land-use planners, policy makers, global travelers, explorers, naturalists, and ecologists to assess the zoonotic and emerging infectious disease hazards at specific locations. The goal was to provide a user-friendly, publicly available resource that simplified the complexity of assessing zoonotic hazards in a particular area, while preserving the scientific foundation required for product utility. Through a systematic and iterative design, PREDICT developed a conceptual framework for zoonotic disease risk assessment culminating in a publicly available online application, HealthMap's PathFinder (<http://healthmap.org/pathfinder>; Wolking et al. 2014; Figure 4).

The PathFinder application enables users to generate a baseline inventory of diseases considered a severe threat to public health and safety for a particular region and highlights zoonotic diseases of concern in an interactive table. Through HealthMap, the application also provides analytical tools (time series analysis, categorization of diseases according to symptoms, the PREDICT Hotspots layer, etc.) to better illustrate the history and potential hazard of disease outbreaks in an area. The tool also has a data export feature allowing users access to the data for further analysis.

PathFinder was designed to support spatially explicit, rapid desktop assessments for zoonotic diseases. If used in tandem with existing environmental and health impact assessment tools

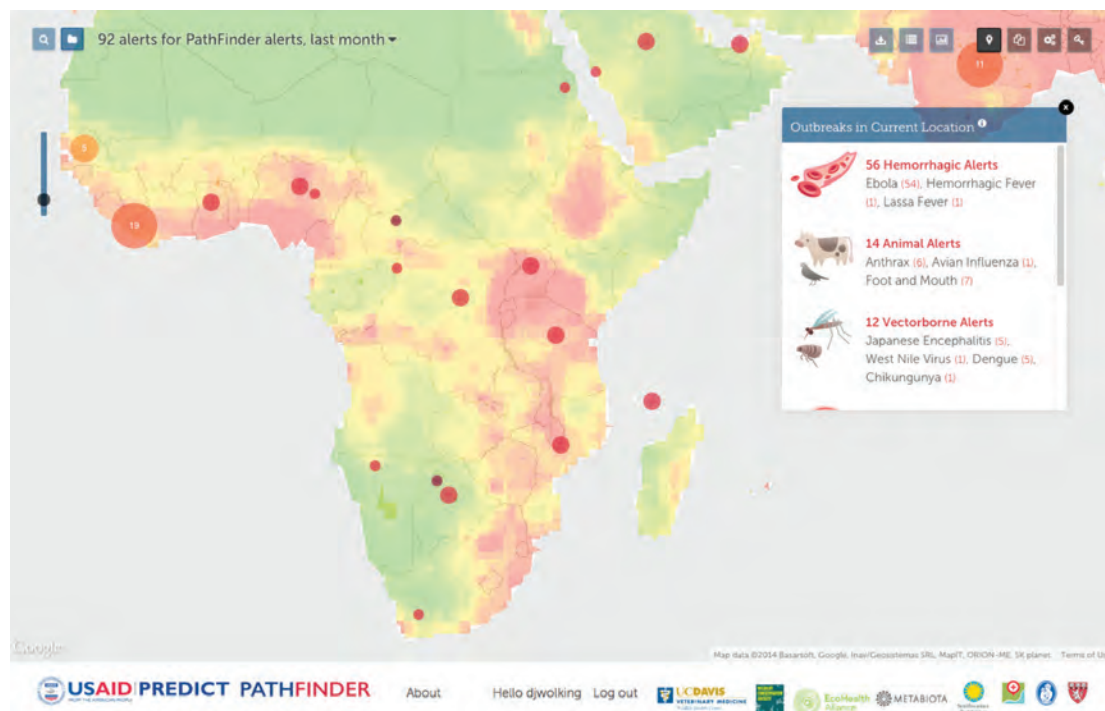


Figure 4. HealthMap PathFinder output for Sub-Saharan Africa showing the PREDICT Hotspot layer of potential disease emergence risk and 92 official reports of zoonotic diseases of concern from the past 30 days (sourced on July 14, 2014). PathFinder (healthmap.org/pathfinder).

widely promoted by the World Bank, International Finance Corporation, and private industry, PathFinder output could equip consultants and assessors with improved real-time data on zoonotic diseases and best estimates for potential risk of disease emergence. Optimally, data and visualization tools from HealthMap PathFinder and other freely available online applications like Map of Life (www.mol.org), which integrates multiple data sets into dynamic species distribution maps, would be combined to enable identification of both disease presence and potential disease distribution by known animal and vector hosts.

Local Media Surveillance (LMS) Study. Although the role of media in the global communication of disease events is becoming increasingly important, not all local media sources are systematically screened using global digital media surveillance, especially missed are those published in local languages. Therefore, PREDICT launched a local media surveillance (LMS) pilot study in seven countries (Bangladesh, Bolivia, Cambodia, Cameroon, Nepal, Tanzania, and Uganda) to monitor disease events in local media sources and assess the value and utility for LMS to enhance surveillance and early recognition of disease events (see Schwind et al. 2014).

A media inventory was gathered at the onset of LMS in each participating country, and media sources were suggested for LMS inclusion by local team members. Selected sources were reviewed by PREDICT to ensure they were not already feeding into main aggregation sites, including Google News or HealthMap, and were important and relevant to the project. Approximately 3-6 local media sources were selected to be scanned each week for health event-related articles by trained participants. Participants in the LMS study included in-country PREDICT personnel who were often able to complete the surveillance during work down time (i.e. drivers while waiting in the field for samples to be collected or laboratory workers awaiting test results from confirmatory laboratories). Participants sent all recorded health events, time spent, and sources surveyed to a central team member in order to determine average weekly surveillance effort. Reports were then sent to HealthMap for inclusion in their digital surveillance system.



PHOTO BY ERIKA ALANDIA

Local newspaper kiosks, such as this one in Bolivia, were surveyed in order to identify appropriate media sources for inclusion in the local media surveillance program.

Over a 16 week evaluation period, the local media health reports were compared to reports in the global HealthMap system to identify unique and overlapping health event coverage. In addition, LMS participants were surveyed to measure attributes of the LMS against the CDC's guidelines for evaluating public health surveillance systems, including simplicity, acceptability, and timeliness.

Local media surveillance filled gaps in digital surveillance network coverage by contributing valuable localized information on risk and disease events to the global HealthMap database, as approximately 67% of all submitted LMS reports from seven participating countries were not captured by HealthMap. Local media surveillance also contributed unique, useful, and critical information on disease events at the local level. The strengths of the LMS were its ease of implementation and minimal resource commitment. Another advantage of LMS was the diverse languages supported, in contrast to digital media surveillance programs that do not currently support all languages. The LMS program evaluation demonstrated that screening local media for health information can be an effective and worthwhile addition to active digital surveillance networks in regions with less-developed capacity for

disease detection and response, even in areas with relatively robust internet connectivity and an abundance of online digital media (see Schwind et al. 2014).

Global Virus Distribution Basemaps. As a component of ongoing information tracking and in an effort to examine the distribution of potential zoonotic threats, PREDICT mapped zoonotic viruses in humans by locality, as documented in the peer-reviewed literature. The effort included mapping of animal-origin viruses that were thought to be associated with infection in humans through PCR, virus isolation, and serologic testing. These virus distribution basemaps were used to inform on sampling and testing and to begin to geographically map the locations of known and new viruses targeted through PREDICT work.

Distributions of individual viruses were mapped using the Geographical Information Systems (GIS) software, ArcGIS 10.2 (ESRI 2012), and then displayed on maps according to their classification by genus. The following maps show examples of seven genera that are globally distributed, such as the Arenaviruses and Flaviviruses, but within them include some viruses with regional distributions. Specific viruses of high consequence, such as Middle Eastern Respiratory Syndrome (MERS) Coronavirus and Severe Acute Respiratory Syndrome (SARS) Coronavirus were mapped to examine local outbreaks and locations of infection, as well as spread to other countries and regions, such as through human travel. A list of references for the 607 citations used to generate these maps is available at: <http://www.vetmed.ucdavis.edu/ohi/predict/virus-references.cfm>.

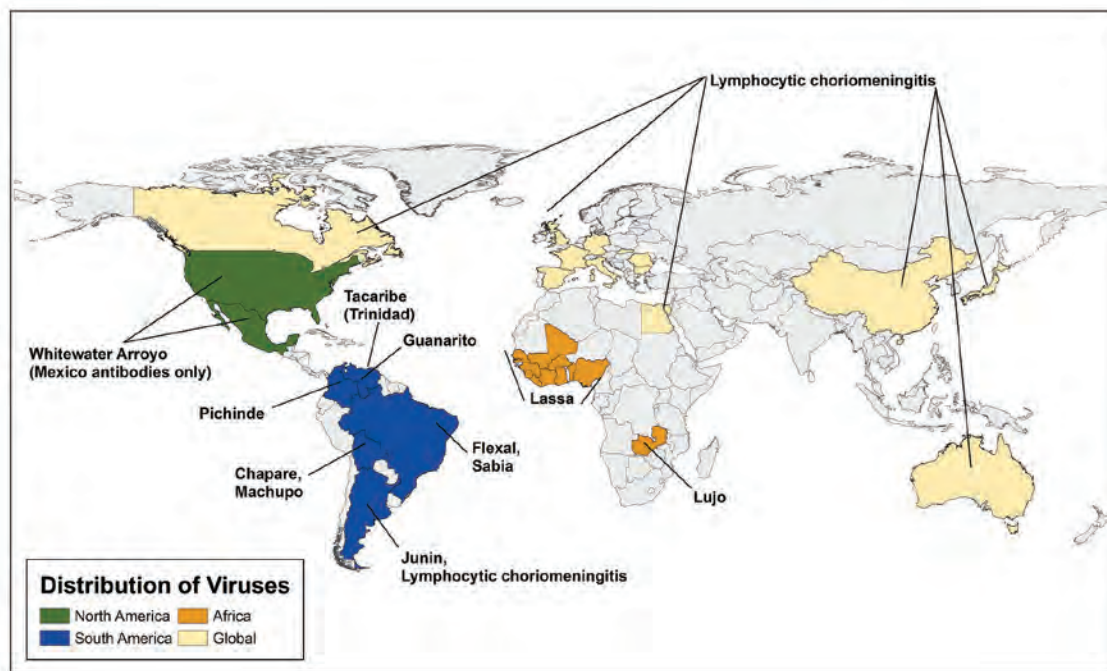


Figure 1: Distribution of viruses within the Arenavirus genus (in the Arenavirus family) associated with human infection as reported by virus and antibody detection in humans.

References: Cassady 1955; Johnson 1965; Sanmartin et al. 1970; White 1972; Buchmeier et al. 1974; Murphy 1975; Wulff & McIntosh 1977; McCormick et al. 1987; Karamany & Imam 1991; Mills et al. 1991; Morita et al. 1991; Tesh et al. 1994; Barry et al. 1995; Ter Meulen et al. 1996; Rousseau et al. 1997; Enria et al. 1998; Marrie & Saron 1998; de Manzione et al. 1998; Weaver et al. 2000; Enria & Pinheiro 2000; García et al. 2000; Asper et al. 2001; Fulhorst et al. 2001, 2002, 2007, 2008; Meulen et al. 2001; Charrel et al. 2001a, 2011; Zhang et al. 2001; Chakarala 2004; Crowcroft et al. 2004; Sottosanti et al. 2005; Chiappero et al. 2005; Omilabu et al. 2005; Macher & Wolfe 2006; Cooke & Shapiro 2006; Lecompte et al. 2006; Becker et al. 2007; Fair et al. 2007; Polop et al. 2007, 2008; Ceianu et al. 2008; Emmerich et al. 2008; Delgado et al. 2008; Ehichioya I et al. 2008; Aguilar et al. 2009b; Ledesma et al. 2009; Cajimat et al. 2009, 2011; Briese et al. 2009; Paweska et al. 2009; Asnis et al. 2010; Ehichioya et al. 2010; Mcconnell et al. 2010; Garcia et al. 2010; Okogbenin et al. 2010; Inizan et al. 2010; Goeijenbier et al. 2013.

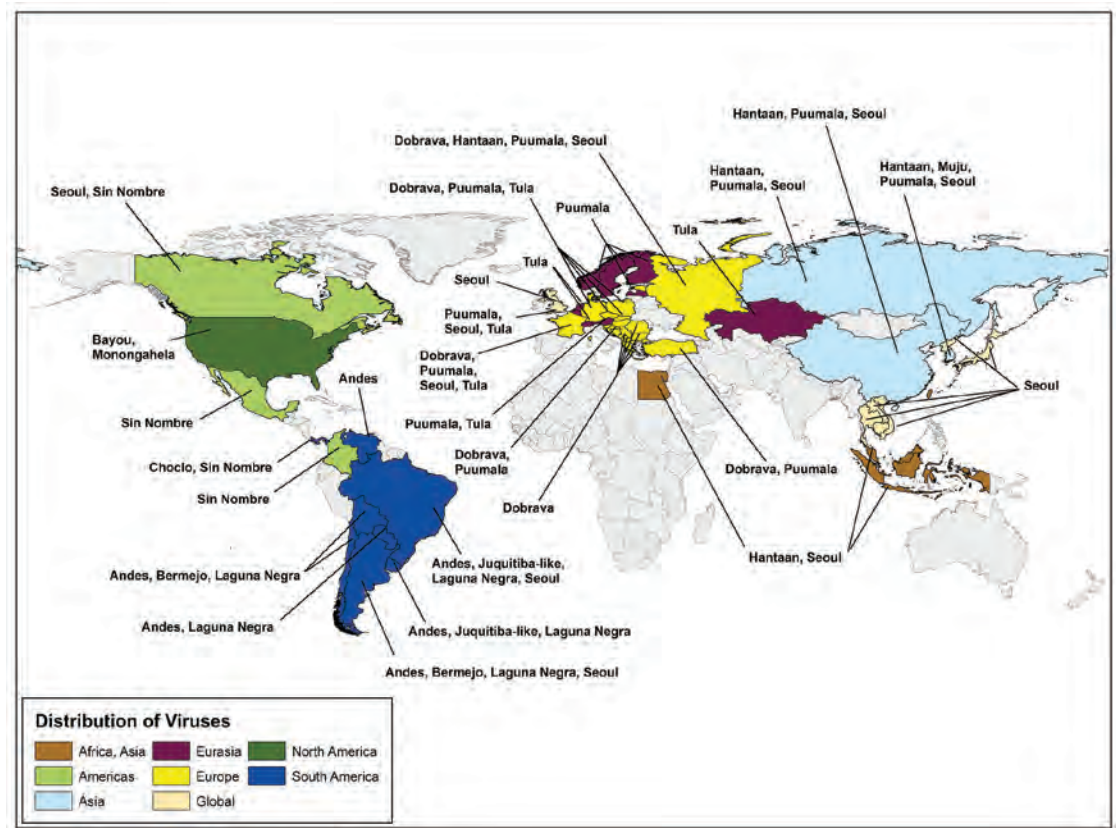


Figure 2: Distribution of viruses within the Hantavirus genus (in the Bunyavirus family) associated with human infection as reported by virus and antibody detection in humans.

References: Chen et al. 1986b; Kao et al. 1996b; Lundkvist et al. 1998b; Plyusnin et al. 1999b; Rhodes et al. 2000b; Ramos et al. 2000b, 2001b; Hjelle & Glass 2000b; Padula et al. 2000b, 2002b; Escutenaire et al. 2000b; Song et al. 2000b, 2004b, 2007b; Castillo et al. 2001b; Papa et al. 2001b, 2010b; Castillo et al. 2004b; Papa et al. 2013b; Passaro et al. 2001b; Heyman et al. 2001b, 2004b; Yahnke et al. 2001b; Galeno et al. 2002b; Nichol et al. 2002b; Schultze et al. 2002b; Gonzalez Della Valle et al. 2002b; Douglass et al. 2003b; Pini et al. 2003b; Riquelme et al. 2003b; Nemirov et al. 2003b; Ferrer et al. 2003b; Clement et al. 2003b; Reynes et al. 2003b; Chu et al. 2003b; Klempa et al. 2004b, 2008b; Mattar & Parra 2004b; Mendes et al. 2004b; Plyusnina et al. 2004b, 2012b; Levis et al. 2004b; Lee et al. 2004b; Suzuki et al. 2004b; Weidmann et al. 2005b; Calisher et al. 2005b; Carroll et al. 2005b; Sironen et al. 2005b; Mailles et al. 2005b; Mathes et al. 2005b; Overturf 2005b; Cvetko et al. 2005b; McIntyre et al. 2005b; Essbauer et al. 2006b; Yan et al. 2007b; Lázaro et al. 2007b; Sinclair et al. 2007b; Abu Sin et al. 2007b; Jakab et al. 2007b; Schilling et al. 2007b; Webster et al. 2007b; Hofmann et al. 2008b; Saksida et al. 2008b; Tersago et al. 2008b, 2011b; Zuo et al. 2008b; Pettersson et al. 2008b; Schmidt-Chanasit et al. 2008b; Zou et al. 2008b; Delfraro et al. 2008b; Uyar et al. 2009b; Ramsden et al. 2009b; Rivers et al. 2009b; Nowakowska et al. 2009b; Zhang et al. 2009b, 2010b; Medina et al. 2009b; Holsomback et al. 2009b; Raboni et al. 2009b; Armién et al. 2009b; Schwarz et al. 2009b; Winter et al. 2009b; Oliveira et al. 2009b; Woods et al. 2009b; Dzagurova et al. 2009b; Kariwa et al. 2009b; Sames et al. 2009b; Schlegel et al. 2009b; Johansson et al. 2010b; Vapalahti et al. 2010b; Cline et al. 2010b; Polop et al. 2010b; Martinez et al. 2010b; Heroldová et al. 2010b; Hukic et al. 2010b; Richter et al. 2010b; Torres-Pérez et al. 2010b; Braun et al. 2010b; Hjertqvist et al. 2010b; Makary et al. 2010b; Nielsen et al. 2010b; Klein et al. 2011b; Milazzo et al. 2012b; Liu et al. 2012b; Cruz et al. 2012b; Smajlović et al. 2012b.

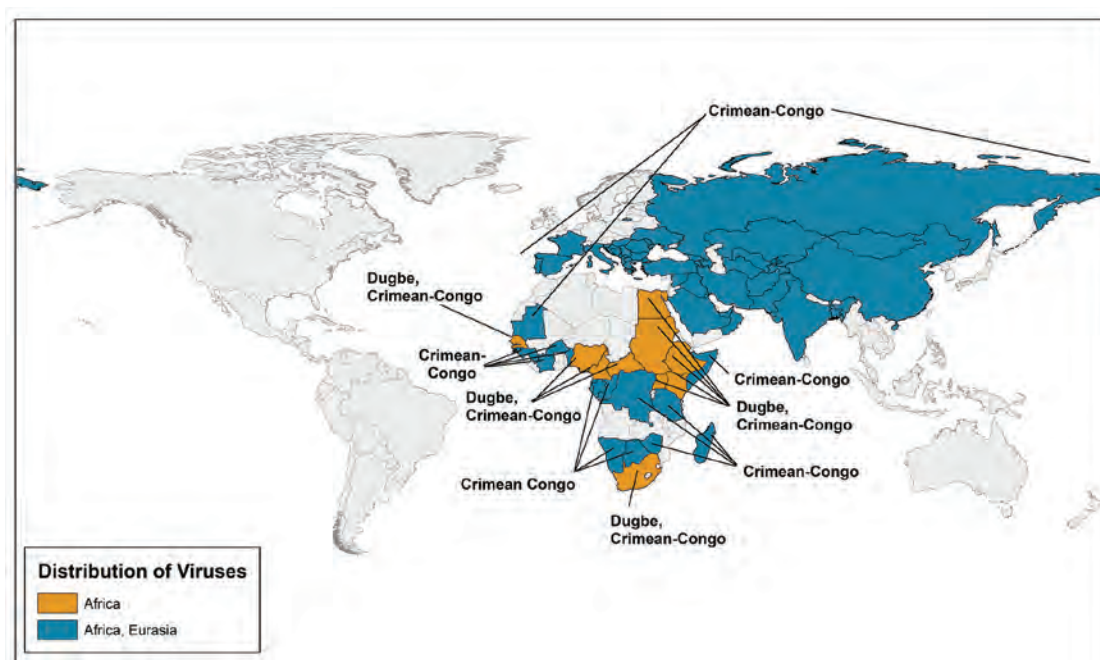
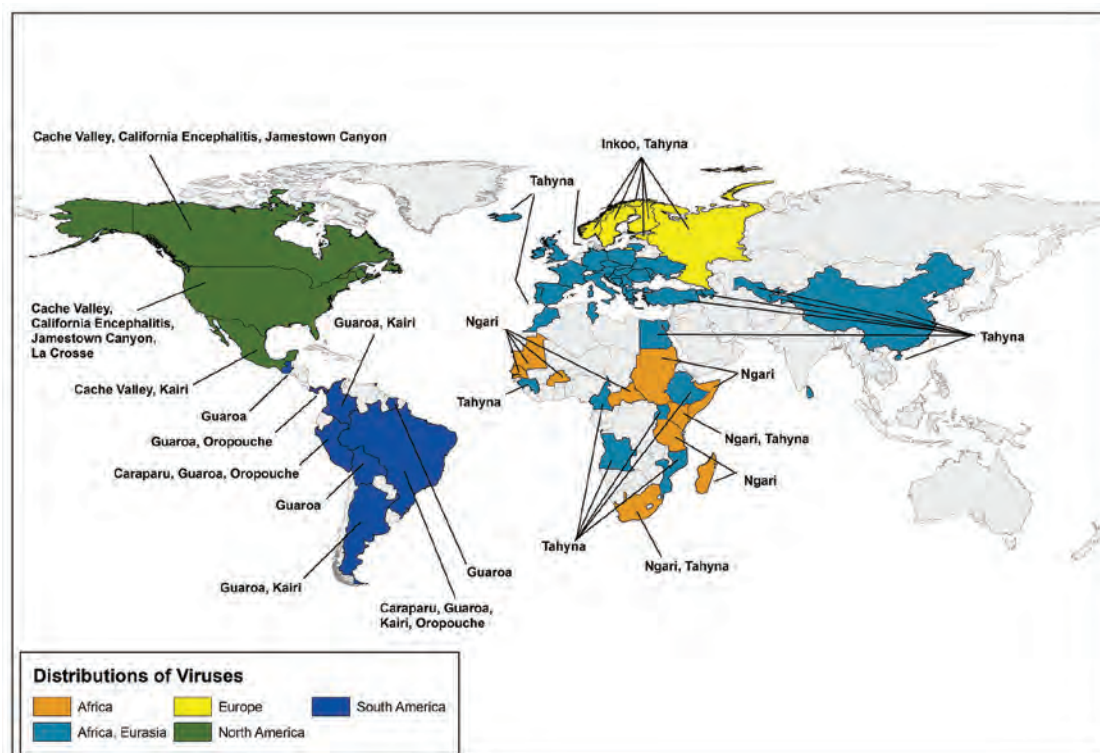


Figure 3: Distribution of viruses within the Nairovirus genus (in the Bunyavirus family) associated with human infection as reported by virus and antibody detection in humans.

References: Wood et al. 1978; Darwish et al. 1983; Gonzalez et al. 1989; Steele & Nuttall 1989; Burt et al. 1996; Drosten et al. 2002; Papa et al. 2002, 2011; Athar et al. 2003; Izadi et al. 2004; Ergönül 2006; Boyd et al. 2006; Ergönül et al. 2006; Nabeth 2007; Midilli et al. 2007; Mardani et al. 2007; Rai et al. 2008; Sharifi-Mood et al. 2008; Gunes 2009; Yilmaz et al. 2009; Sun et al. 2009a; Ertugrul et al. 2009; Vorou 2009; Günaydin et al. 2010; W & Waterman 2010; Aradaib et al. 2010; Carroll et al. 2010; Tezer et al. 2010; Patel et al. 2011; Andriamandimby et al. 2011; Goel et al. 2012; Lindeborg et al. 2012; Ergonul 2012.



References: Dick 1953; Aitken et al. 1964; Kokernot et al. 1969; Buescher et al. 1970; Issel et al. 1973; Gauld et al. 1974; Balfour et al. 1976; Pennington et al. 1977; R.S. 1981; Calisher et al. 1983, 1986, 1988; Zarnke et al. 1983; Bárdos et al. 1983; Grimstad et al. 1986; Eldridge et al. 1987, 2001; McLean et al. 1987; Stansfield et al. 1988; Butenko et al. 1991; Neitzel & Grimstad 1991; Aguirre et al. 1992; Madic et al. 1993; Leber et al. 1995; Sexton et al. 1997; Blackmore & Grimstad 2008, 1998; Rust et al. 1999; Sahu et al. 2000, 2002; Miller et al. 2000; Gerhardt et al. 2001; Mayo et al. 2001; Reisen et al. 2001, 2004; Sokol et al. 2001; Concha-Bermejo 2003; Trevejo 2004; Nelson et al. 2004; Gerrard et al. 2004; Mercer & Castillo-pizango 2005; Nunes et al. 2005c, 2005a; Gould et al. 2006a; Campbell et al. 2006; Kuniholm et al. 2006; Armstrong & Andreadis 2006, 2007; Medlock et al. 2007; Zheng et al. 2007; Putkuri et al. 2007; Andreadis et al. 2008; N. Putkuri I., A. Vaheri I. 2008; Hubálek 2008; Haddow & Odoi 2009; Soto et al. 2009; Lu et al. 2009, 2011; Tauro et al. 2009; Terzian et al. 2009; Alatoon & Payne 2009; Wang et al. 2009; Forshey et al. 2010; Murdock et al. 2010; Kilian et al. 2010; Jentes et al. 2010; Aguilar et al. 2010; Report 2011; Haddow et al. 2011; Blitvich et al. 2012; Goff et al. 2012; Holden & Hess 2013; Morgante & Shernanchuk 2013; Reeves & Miller 2013

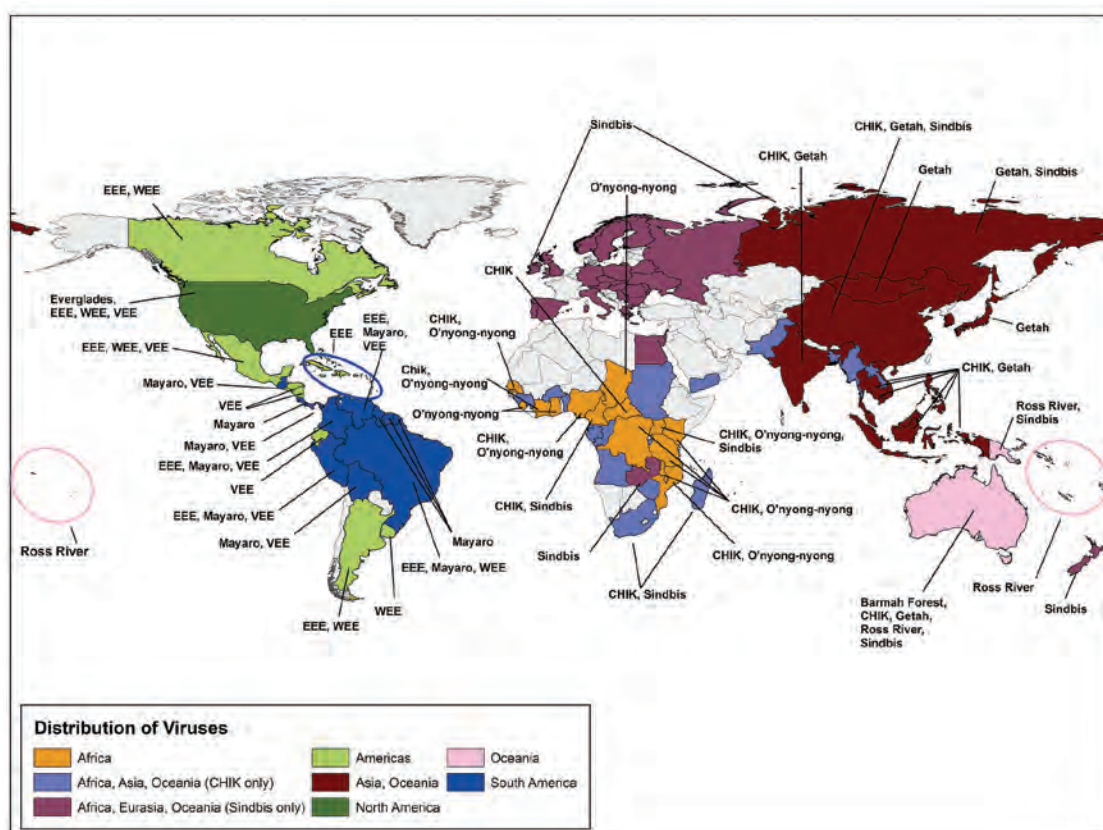


Figure 7: Distribution of viruses within the Alphavirus genus (in the Togavirus family) associated with human infection as reported by virus and antibody detection in humans.

References: Lumsden 1955; Bradish et al. 1971; Calisher et al. 1980; Cross et al. 1981; Maness & Calisher 1981; Johnson et al. 1981; Marshall & Woodroffe 1982; Standfast et al. 1984; Vale et al. 1986; Lindsay et al. 1995; Rico-Hesse et al. 1995; Eisenhut et al. 1999; Samuels et al. 1999; Atkins et al. 1999; Bi et al. 2000; Hommel et al. 2000; Luukkainen et al. 2000; Powers et al. 2000, 2006; Harley et al. 2001; Brummer-Korvenkontio et al. 2002; Jeffery et al. 2002, 2006; Ferro et al. 2003; Thoisy et al. 2003; JR et al. 2004; Kelly-hope et al. 2004; Tong 2004; Weaver et al. 2004; Laine et al. 2004; Coffey & Weaver 2005; Johansen et al. 2005; Klapsing et al. 2005; Quinn et al. 2005; Navarro et al. 2005; Loftin et al. 2006; Bessaud et al. 2006; Coffey et al. 2006; Abraham & Sridharan 2007; AbuBakar et al. 2007; Rezza et al. 2007; Hachiya et al. 2007; Aguilar et al. 2007, 2009a; Kay et al. 2007; Powers & Logue 2007; Vlen et al. 2007; Bonilauri et al. 2008; Nero 2008; Kurkela et al. 2008; Morrison et al. 2008; Pierro et al. 2008; Jacups et al. 2008; Zhai et al. 2008; Forrester et al. 2008; Azevedo et al. 2009; Chahar et al. 2009; Harvala et al. 2009; Hossain et al. 2009; Juricova et al. 2009; Sun et al. 2009b; Quiroz et al. 2009; Naish et al. 2009; Auguste et al. 2009, 2010a; Tappe et al. 2009; Gasque et al. 2010; Hassing et al. 2010; Armstrong & Andreadis 2010; Thiboutot et al. 2010; Zacks & Paessler 2010; Gaibani et al. 2010; Hapuarachchi et al. 2010; Dwibedi et al. 2010, 2011; Receveur et al. 2010; Das et al. 2010; Rianthavorn et al. 2010; Manimunda et al. 2010; Ayu et al. 2010; Soumahoro et al. 2010; Delfraro et al. 2011; Mutebi et al. 2011; Tan et al. 2011; Abad-Franch et al. 2012; Gobbi et al. 2012; Neumayr et al. 2012; Wu et al. 2012; Adams et al. 2012; Uejio et al. 2012; Kucharz & Cebula-Byrska 2012; Zayed et al. 2012; Emmons & Lennette 2013; Tully et al. 2013; Williams et al. 2013.

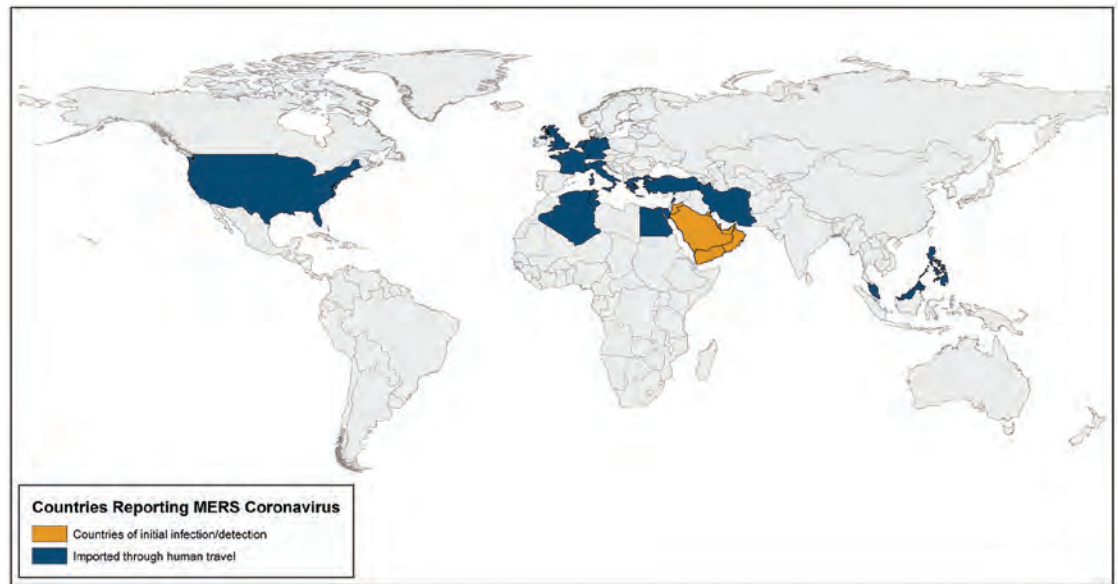


Figure 8: Countries reporting Middle Eastern Respiratory Syndrome (MERS) Coronavirus (within the Coronavirus family) infections in people.

References: Puzelli et al. 2013; Arabia 2014; Briese et al. 2014; Jawf et al. 2014; Nowotny & Kolodziejek 2014; Kraaij–Dirkzwager et al. 2014; Reusken et al. 2014.

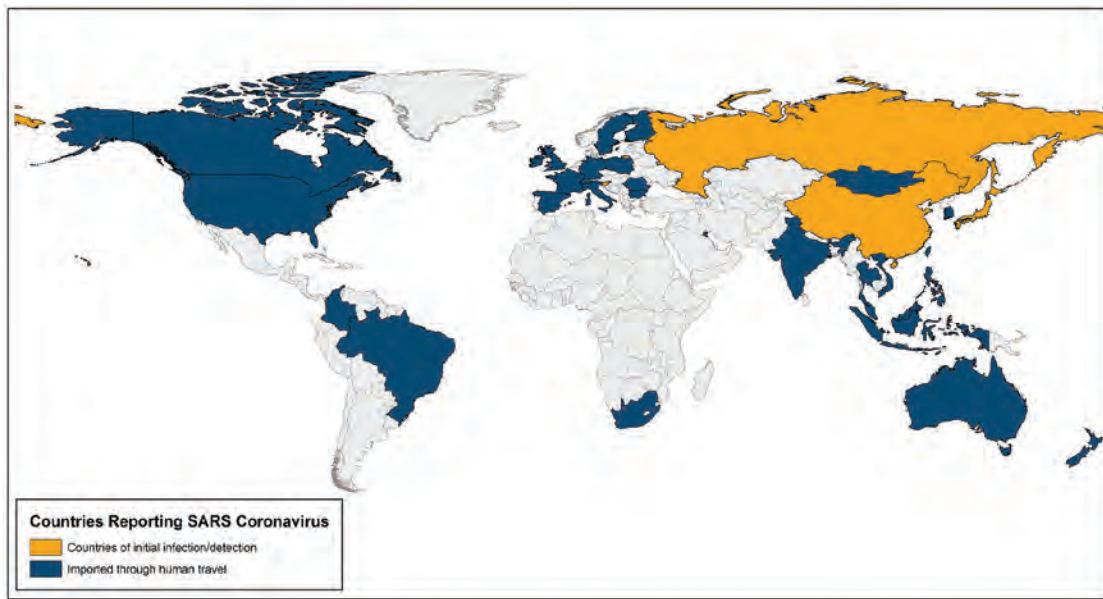


Figure 9: Countries reporting Severe Acute Respiratory Syndrome (SARS) Coronavirus (within the Coronavirus family) infections in people.


References: WHO 2003; Boulos 2004; Wang et al. 2005, 2006; Wang & Eaton 2007; Shi & Hu 2008; Tong et al. 2009; Field 2009; Rihtaric et al. 2010.

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CAPACITY BUILDING





A key focus of PREDICT was local capacity building to improve wildlife surveillance in hotspot regions where emergence of pathogens of wildlife origin was mostly likely to occur. PREDICT specifically sought to operationalize One Health by helping to improve local, regional, and global surveillance networks for detection of viruses in wildlife and for outbreak prevention and response.

Examples of PREDICT capacity building efforts included transfer of technologies and testing protocols to local and regional laboratories; facilitating equipment acquisition to improve cold chain and laboratory infrastructure; participating in local and regional training workshops to enhance personnel knowledge and skills; providing on-the-job training in field sampling, safe collection and handling of specimens, and laboratory techniques; facilitating working groups to bring field and laboratory personnel together for increased collaboration; disseminating detailed protocols for all aspects of surveillance activities; and developing integral partnerships with local organizations for the advancement of the program objectives. Capacity building methodology for PREDICT focused on two main objectives: 1) determining the baseline understanding of issues and local capabilities and its change over time for wildlife zoonotic virus surveillance in each participating country, and 2) utilizing information gathered in order to target training and needs for infrastructure improvement (i.e. human resources, laboratory resources, disease surveillance, and One Health partnerships).

Tracking of changes in wildlife surveillance competencies helped to target capacity building priorities on both national and regional levels. Using a rapid survey tool that was developed internally, PREDICT Country Coordinators tracked in-country capabilities and changes over time for wildlife surveillance, zoonotic virus detection, and assistance with outbreak response. This process served to inform on and focus internal project planning efforts. Specifically, data concerning key measures of current activities, including laboratory protocol tracking, disease surveillance, outbreak response, and One Health actions, as well as challenges in conducting and opportunities for improving wildlife virus detection, were gathered in order to better understand activities in each region where the PREDICT project was active (also see Schwind et al. 2014).

In the first and second years, the focus was on understanding the baseline activities and efforts related to the monitoring of wildlife for zoonotic viruses. Therefore, the PREDICT survey tool was divided into five core areas: 1) general efforts, 2) human resources, 3) laboratory resources, 4) surveillance and outbreak response for zoonotic diseases, and 5) risk assessment and communication. Each Country Coordinator was instructed to complete the survey using individual knowledge, publically available information, and/or information from applicable contacts familiar with the topic areas. Country Coordinators were also encouraged to only provide information for areas on which they were sufficiently knowledgeable or where specific sources of information could be cited, thus the data was not comprehensive for all countries.

For years three through five, in addition to completing the rapid survey tool, which continued to track efforts in each of the aforementioned core areas of focus, Country Coordinators were also asked to identify at least one key stakeholder within their country to interview to assess the external perspective on the role the project played in improving capacity for wildlife sampling and zoonotic virus detection. Examples of key stakeholders included officials from government ministries and wildlife management or conservation departments. Questions were asked regarding the challenges, opportunities, and priorities relevant to wildlife surveillance for zoonotic pathogens. Stakeholder input was of interest to determine external perspectives on the PREDICT project and associated capacity building efforts. By not solely relying on the PREDICT Country Coordinators' perceptions, the team was able to develop a more comprehensive picture of perspectives regarding wildlife surveillance to detect zoonotic viruses and the role PREDICT played in improving capacity to perform this work in each country. The completed surveys were collated, analyzed, and used to identify challenges and opportunities associated with conducting wildlife virus surveillance, potential linkages across sectors that could help operationalize One Health, and changes in capacity over time in each country.

HIGHLIGHTS FROM THE SURVEY TOOL

Key Measures

Core Capacities. To characterize the impact of PREDICT on core capabilities, Country Coordinators identified, in the final rapid survey tool, the areas specifically improved due to PREDICT activities during the five-year period. Core capacities examined through this survey included wildlife field surveillance protocols, trained field surveillance and laboratory personnel, use of effective cold chain, laboratory diagnostic protocols, data management, intersectoral cooperation, outbreak response, inclusion of wildlife in outbreak response activities, and implementation of government programs and policies. The number of countries by region that indicated improvement specifically due to the PREDICT project in each of these core areas is shown in Figure 1. The greatest increase in core capabilities, which was observed across all countries, was through the training of field surveillance personnel. One-hundred percent (n=19) of reporting PREDICT countries stated that improvement was seen in the number of skilled workers available to conduct surveillance activities in-country. This top improvement was followed by an overall increase in the availability of both laboratory diagnostic protocols (n=18) and wildlife field surveillance protocols (n=18) in PREDICT countries.

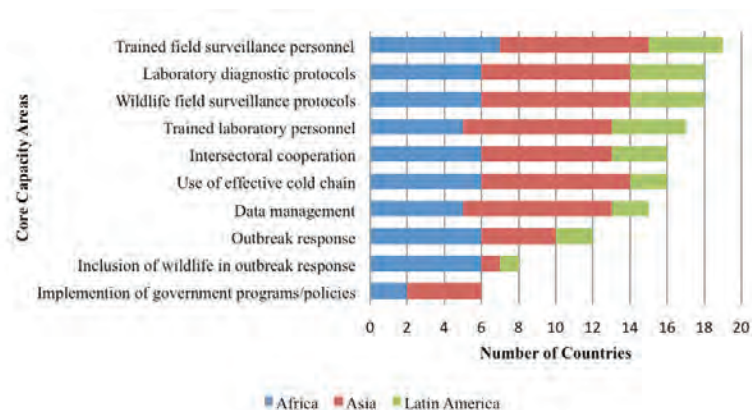


Figure 1. Number of countries by region where improvement in core activity areas was documented through PREDICT (n=19).

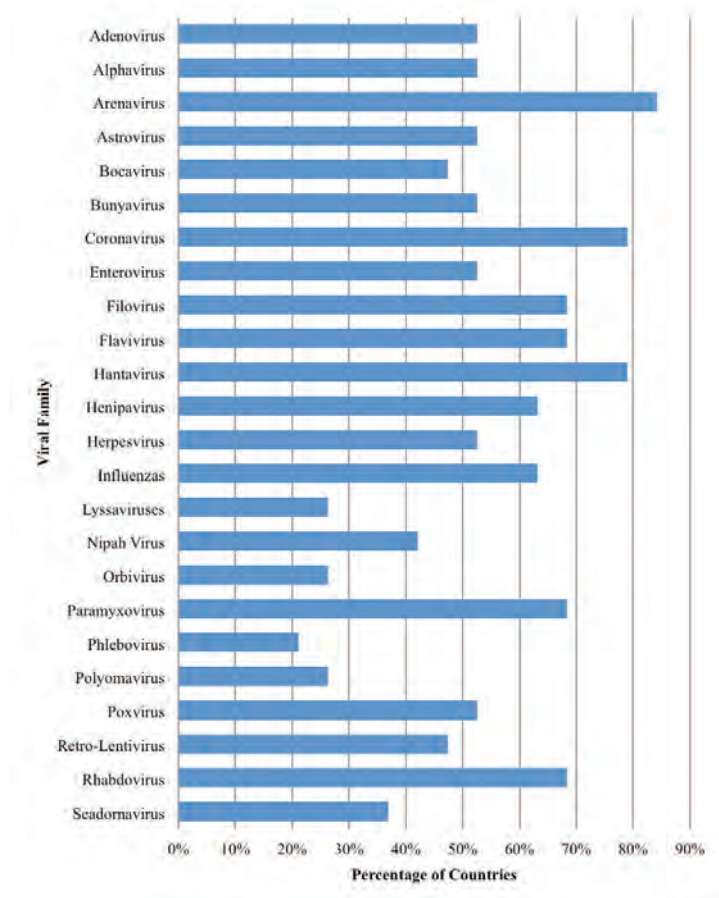


Figure 2. Percentage of countries where viral family testing protocols were performed in the final year of the PREDICT project.

Laboratory Protocol Tracking. A key area of capacity building in global hotspot regions was the implementation of viral family PCR testing protocols in-country. The percentage of countries that implemented PREDICT testing protocols by viral family is presented in Figure 2. Seventeen (90%) of PREDICT countries reported performing at least one PREDICT viral family testing protocol in-country by the final year. In addition, three (43%) countries in Africa, four (50%) countries in Asia, and two (50%) countries in Latin America had at least one PREDICT viral family testing protocol being utilized by a government laboratory.

Disease Surveillance. Increasing and tracking wildlife sampling efforts at key animal-human interfaces was a specific focus for capacity building throughout all PREDICT countries. In each rapid tool survey, Country Coordinators reported on high-risk disease transmission interfaces where PREDICT was sampling wildlife (see Surveillance Strategy section). Table 1 reports the active animal-human interfaces with PREDICT efforts by region at the end of the fifth year. Areas where animals' primary locations were in or around human dwellings were active wildlife sampling sites across all regions (n=18). In Africa, all seven countries (100%) also reported sampling at hunting interfaces. In Asia, areas where animals came in contact with tourists were a focus for six (75%) countries in the region. In Latin America, all reporting countries in the region also focused efforts in areas where animals came in contact with park personnel and researchers.

Table 1. Most sampled high-risk disease transmission interfaces in PREDICT countries by region.

Africa (n=7)	Asia (n=8)	Latin America (n=4)
In and around human dwelling(s) 7 (100%)	In and around human dwelling(s) 7 (88%)	In and around human dwelling(s) 4 (100%)
Hunted/consumed 7 (100%)	Ecotourism 6 (75%)	Contact with wildlife personnel/ intensive wildlife management area 4 (100%)
Contact in agricultural fields 5 (71%)	Market/trade 4 (50%)	Contact with researchers (other than PREDICT staff) 4 (100%)
Ecotourism 5 (71%)	Contact in agricultural fields 4 (50%)	Contact with domestic animals* or humans NOT likely 4 (100%)
Market/trade 4 (57%)	Zoos 4 (50%)	Sanctuaries 3 (75%)
Zoos and Sanctuaries 4 (57%)	Sanctuaries 4 (50%)	Contact in extractive industry 3 (75%)
Contact with researchers (other than PREDICT staff) 4 (57%)	Contact with locals for religious activities 4 (50%)	Contact in agricultural fields 3 (75%)

* livestock, dogs and cats

Outbreak Response. Assistance by PREDICT in disease outbreak response was also tracked through the rapid tool survey. These outbreaks included suspected or confirmed cases of brucellosis, Chikungunya, Ebola, encephalomyocarditis virus, Marburg, Monkeypox, Rift Valley fever, viral hemorrhagic fever, and yellow fever in Africa; avian influenza, gammaherpesvirus, hepatitis, Japanese encephalitis, malaria, Nipah virus, and sarcocystis in Asia; and rabies, viral hemorrhagic fever, and yellow fever in Latin America. The extent of involvement by PREDICT varied with each outbreak, but Country Coordinators provided various forms of assistance, such as technical advice and expertise to help in designing response strategies, animal sampling in the field, processing of samples in local laboratories, epidemiologic risk behavior surveys, and training of personnel employed by partner organizations in PREDICT surveillance protocols during outbreaks.

One Health Actions. One Health concepts were operationalized through PREDICT in numerous ways since the onset of the project. PREDICT teams regularly participated in One Health workshops, conferences, and meetings with strategic partners; fostered coordinated surveillance and outbreak investigations with local government ministries; built new bridges between wildlife and public health authorities through the development of outbreak response protocols; and raised awareness of wildlife health among key central and provincial government officials from several relevant One Health disciplines (e.g. animal health, human health, forestry, environment, and education). Types of efforts that were reported through the rapid tool survey in the last year of the project included coordinated response efforts, improvement of local

Table 2. Examples of the operationalization of One Health concepts through PREDICT activities.

Topic	Example
Coordinated response efforts	<p><i>"Two outstanding examples of how the One Health approach was operationalized in the country are the response activities to the fox-rabies and the yellow fever outbreaks. Prompt technical advice, investigations, and response activities prevented massive persecution and hunting of foxes along with the consequent ecosystem negative effects associated with fox eradication, and also prevented yellow fever infections in humans."</i></p> <p>– Country Coordinator; Bolivia</p>
Improvement of local capacity	<p><i>"Local capacity for wildlife disease surveillance was improved through the implementation of training and introduction of PREDICT field protocols. Now, the national reference laboratory implements pathogen screening on wildlife samples to detect zoonotic viruses and is sharing archived and newly collected samples with PREDICT for expanded pathogen testing during outbreak response."</i></p> <p>– Country Coordinator; Peru</p>
Increased educational opportunities	<p><i>"There have been ongoing One Health trainings and courses at different institutions that link participants from different professional backgrounds. One example would be the master's degree program on One Health in Molecular Biology at Sokoine University of Agriculture."</i></p> <p>– Country Coordinator; Tanzania</p>
Increased intersectoral communication	<p><i>"PREDICT Nepal initiated a One Health approach in Nepal by supporting the "One Health Workshop" and One Health Alliance Nepal (OHAN) in Nepal for the first time. This organization is working on lobbying for the establishment of a One Health secretariat in Nepal to solve the problem of authority over research and outbreaks related to zoonotic diseases."</i></p> <p>– Country Coordinator; Nepal</p> <p><i>"Quarterly meetings among human health, livestock health, and wildlife health have led to increased sharing of knowledge and collaboration on lab capacity building and surveillance activities."</i></p> <p>– Country Coordinator; Laos</p>
Increased awareness regarding the importance of wildlife	<p><i>"Veterinary schools in our country are recognizing the importance of a One Health approach, have held One Health workshops, and have invited the forestry, wildlife, domestic animal, and human health sectors to participate in the workshops and in development of their new curricula."</i></p> <p>– Country Coordinator; Cambodia</p>

surveillance capacity, increased educational opportunities, increased intersectoral communication, and increased awareness regarding the importance of wildlife. Table 2 provides specific examples of applications of the One Health approach in participating countries.

Global Challenges and Opportunities in Wildlife Pathogen Surveillance

Because research regarding the capacity to conduct zoonotic pathogen surveillance in wildlife on a global scale is nominal compared to its human and domestic animal counterparts, two focused projects were conducted through the PREDICT capacity tracking efforts in order to 1) evaluate baseline trends in wildlife surveillance in PREDICT countries at the onset of the project, and 2) determine similarities and differences in perceptions regarding the current state of wildlife pathogen surveillance between in-country stakeholders and PREDICT Country Coordinators. Major findings from each of these research studies include:

Baseline Capacity Findings. Analysis of expert opinion on baseline capacity showed a lower overall capacity for conducting zoonotic disease surveillance in wildlife compared to livestock/ domestic animal and human zoonotic disease surveillance capacities. Additionally, better wildlife surveillance capacity was not associated with higher income countries contrary to reports in other health sectors. Reported overall surveillance capacity in Africa was lower for both wildlife surveillance and livestock surveillance compared to human surveillance capacity, whereas Asia and Latin America surveillance capacity was lowest for wildlife, somewhat better for livestock, and

was highest for human surveillance. Across the three regions, the highest surveillance capacities across health sectors were reported for Latin America, then Asia, and lastly Africa.

Furthermore, challenges identified by in-country personnel for conducting wildlife surveillance that were categorized as 'Very Important' by at least 50% of countries in each global region were reported in the initial PREDICT capacity assessment. At the beginning of the project, the Country Coordinators' top ranked challenge in all regions was associated with 'Financial/funding issues' (85%); the second highest ranked challenge was 'Insufficient training to work with wildlife' (80%); and the third ranked challenge was associated with 'Poverty issues' (70%). Other challenges included 'Time delay between disease or outbreak events and when official information is publicly available' (65%); 'Lack of existing government wildlife surveillance program' (65%); and 'Insufficient enforcement' (65%).

The project opportunities relating to wildlife surveillance identified as 'Very Important' by at least 50% of participating in-country experts were also reported. The top ranked opportunities for conducting wildlife surveillance for zoonotic pathogens in all regions at the beginning of the PREDICT project were 'Increasing funding availability' (80%); 'Increasing human capacity from new/existing training programs' (80%); and 'Increasing communication/coordination among sectors or agencies' (80%). The second highest opportunities highlighted as "Very Important" included 'Showing usefulness to help integrate wildlife surveillance into program/country infrastructure' (75%) and 'Collaboration with foreign universities/programs/NGOs' (75%). These opportunities were followed by 'Increasing laboratory capacity from existing programs and new facilities' (70%); 'Establishing wildlife policies and programs' (70%); and 'Growing interest/awareness regarding disease issues' (70%).

Establishing baseline capacity across all health sectors (e.g. public health, domestic animal health, and wildlife health) in PREDICT participating countries, as well as understanding challenges and opportunities present in wildlife pathogen surveillance, provided a clearer picture of in-country capabilities for addressing emerging health threats at the onset of the PREDICT project. Study

findings from the baseline capacity surveys highlighted the need for strategic capacity building to focus and facilitate long-term wildlife surveillance efforts at interfaces where new pathogens are most likely to emerge.

Stakeholder Capacity Findings. Once baseline capacity was qualitatively described in each participating country, the rapid tool survey added key stakeholders from outside the PREDICT project beginning in the third year. Administering the rapid tool survey to Country Coordinators as well as wildlife officials was useful to gain diverse insights and perspectives into capacity building for wildlife pathogen surveillance. Both stakeholder groups identified some high-risk disease transmission interfaces, such as wildlife hunting and markets, as important for ongoing targeting of wildlife surveillance.



PHOTO BY MELINDA ROSTAL

PREDICT teams benefit from a wide range of government and scientific partnerships: a wildlife sampling training was held with Indonesia partners in 2012.

Similarly, findings regarding challenges across stakeholder groups showed some agreement in that a lack of sustainable funding across regions was the greatest challenge for conducting wildlife surveillance for zoonotic pathogens (wildlife officials: 96% and Country Coordinators: 81%). However, the opportunity for improving zoonotic pathogen surveillance capacity identified most frequently by wildlife officials as important was increasing communication or coordination among agencies, sectors, or regions (100% of wildlife officials), whereas the opportunities identified most frequently by Country Coordinators as important were increasing human capacity, increasing laboratory capacity, and the growing interest or awareness regarding wildlife disease or surveillance programs (all identified by 69% of Country Coordinators).

PREDICT provided a unique opportunity to explore the capacity building conditions present in wildlife pathogen surveillance in global hotspot regions from the perspectives of people with different experiences and backgrounds. While the differences in stakeholder groups' perspectives regarding the relative importance of challenges and opportunities could be due to differences seen in each organization's current focus, priorities, and mandates; limitations in each individual's area of expertise; or the nature of the survey, the findings provided important insights into stakeholder group views as key informants. The broader surveys illustrated the value of including a variety of in-country people with different backgrounds in order to combat emerging infectious diseases.

Future Directions for Capacity Building and Tracking

Information obtained through the rapid tool surveys and the aforementioned focused projects, contributes to the growing knowledge concerning successful capacity building practices aimed at improving zoonotic pathogen surveillance in locations considered to be hotspots for emergence of animal viruses in human populations. Utilizing key subject matter experts and other stakeholders to identify and prioritize future research directions had many benefits for sustainable capacity building efforts in developing regions. Furthermore, a One Health approach to capacity building for virus detection applied at local and global scales will have a powerful impact on improving zoonotic virus surveillance in wildlife. The PREDICT project put this knowledge into action by placing in-country personnel, who were also wildlife stakeholders, in key programmatic positions which allowed them to demonstrate value to local public health, agriculture and environmental authorities, and stakeholders. In addition, PREDICT used One Health concepts as a guiding force to increase communication and collaboration across governmental ministries and private sectors in-country. Moving forward, the iterative evaluation of One Health activities, such as through the use of rapid tool surveys, should be continued for the promotion of successful, evidence-based practices.

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OUTBREAK RESPONSE



Out-break /'out,brāk/

The sudden or violent start of something unwelcome.

In late March 2014, the World Health Organization and Ministry of Health in Guinea reported an outbreak of Ebola virus disease (EVD) in four southeastern districts. Reports of suspected cases in the neighboring countries of Liberia and Sierra Leone were already under investigation. This was the first reported outbreak of EVD in this area of West Africa.

At the end of September 2014, the Ebola virus had infected over 6,000 individuals in five West African countries and caused over 2,900 deaths (cases and deaths continued to increase past the end-date of PREDICT's first phase). This epidemic has overwhelmed vulnerable health systems and demonstrated pandemic potential catalyzing an international response.

In late August 2014, the Ministry of Health in the Democratic Republic of Congo notified the World Health Organization of a separate Ebola virus disease outbreak in Equateur Province. This was the seventh outbreak of EVD in the country since 1976. A rapid response effort was implemented along with PREDICT staff, and the outbreak was largely contained, in part due to a public sensitized and educated on Ebola from previous outbreaks, but also due to the speed of detection from knowledgeable diagnosticians and rapid case identification and isolation and quarantine of potential contacts, along with the geographic good fortune that the outbreak emerged in a remote area with little connection to transport networks and densely populated urban centers.

Both outbreaks were likely caused by the spillover of Ebola virus into people from a wildlife host. In both outbreaks the virus was then transmitted between people infecting family members, friends, and health care providers. But in one outbreak, a health system was equipped and prepared to implement rapid response and control measures.

Prevention and early control of outbreaks is key to reducing their impact.

There is considerable uncertainty in predicting when and how a virus will spillover from wildlife into domestic animal or human populations. PREDICT's surveillance and capacity building strategy emphasized the detection of pathogens early at their source, before they have the opportunity to emerge or spread from wildlife to people and then amplify in human populations. PREDICT's capacity building strategy encouraged partnerships with governments and local institutions to strengthen disease surveillance and diagnostic centers and improve their capacity to detect known and novel pathogens. In coordination with USAID EPT partners, PREDICT's outbreak response strategy adapted to stakeholder needs to provide requested and critical support in disease investigations, diagnostics, supply procurement, and communications in outbreaks affecting human, domestic animal, and wildlife populations. Further, as outbreak situations often encourage interagency and transdisciplinary cooperation, PREDICT and EPT partners worked with key stakeholders to operationalize the One Health approach, incorporating wildlife and animal population surveillance into disease investigations and promoting the expansion of a trained One Health workforce for response activities.



PHOTOS BY KEVIN OLIVAL



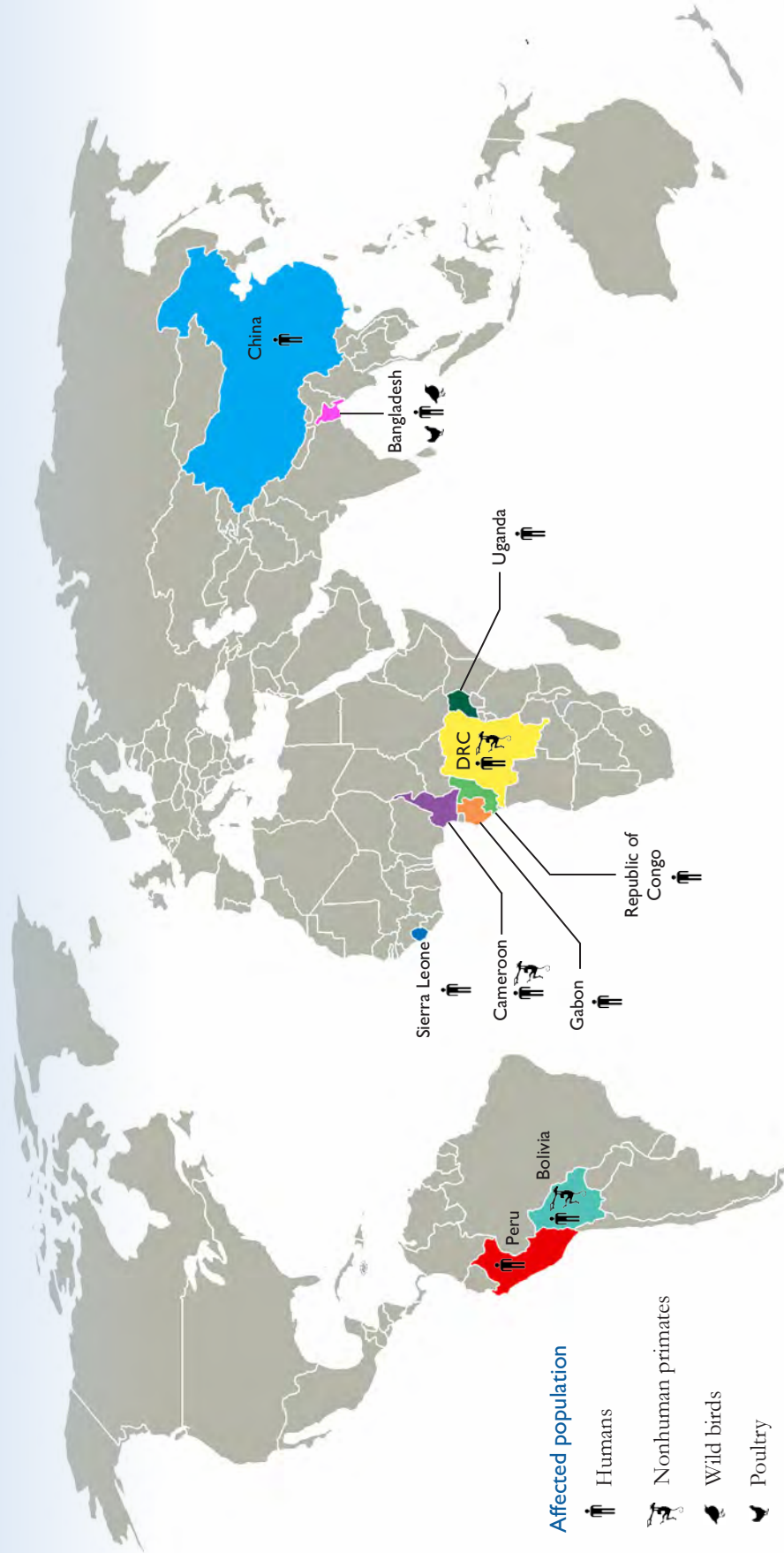
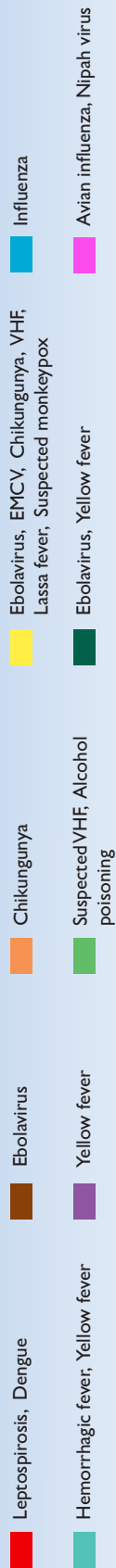
Preparedness workshop for MERS-CoV investigations and surveillance in livestock at the Dusit Zoo in Bangkok. Participants received hands-on training on use of personal protective equipment (PPE) and sample collection from camels.

Between June 2010 and September 2014, PREDICT provided support to lead government and international organizations during 23 outbreaks in ten countries. Of these 23 outbreaks, over 83% involved support to response teams for diseases impacting human populations. PREDICT also assisted response teams during four epidemics involving wildlife populations (chimpanzees, bonobos, red howler monkeys, and wild birds), and two epidemics in domestic animal populations (poultry).

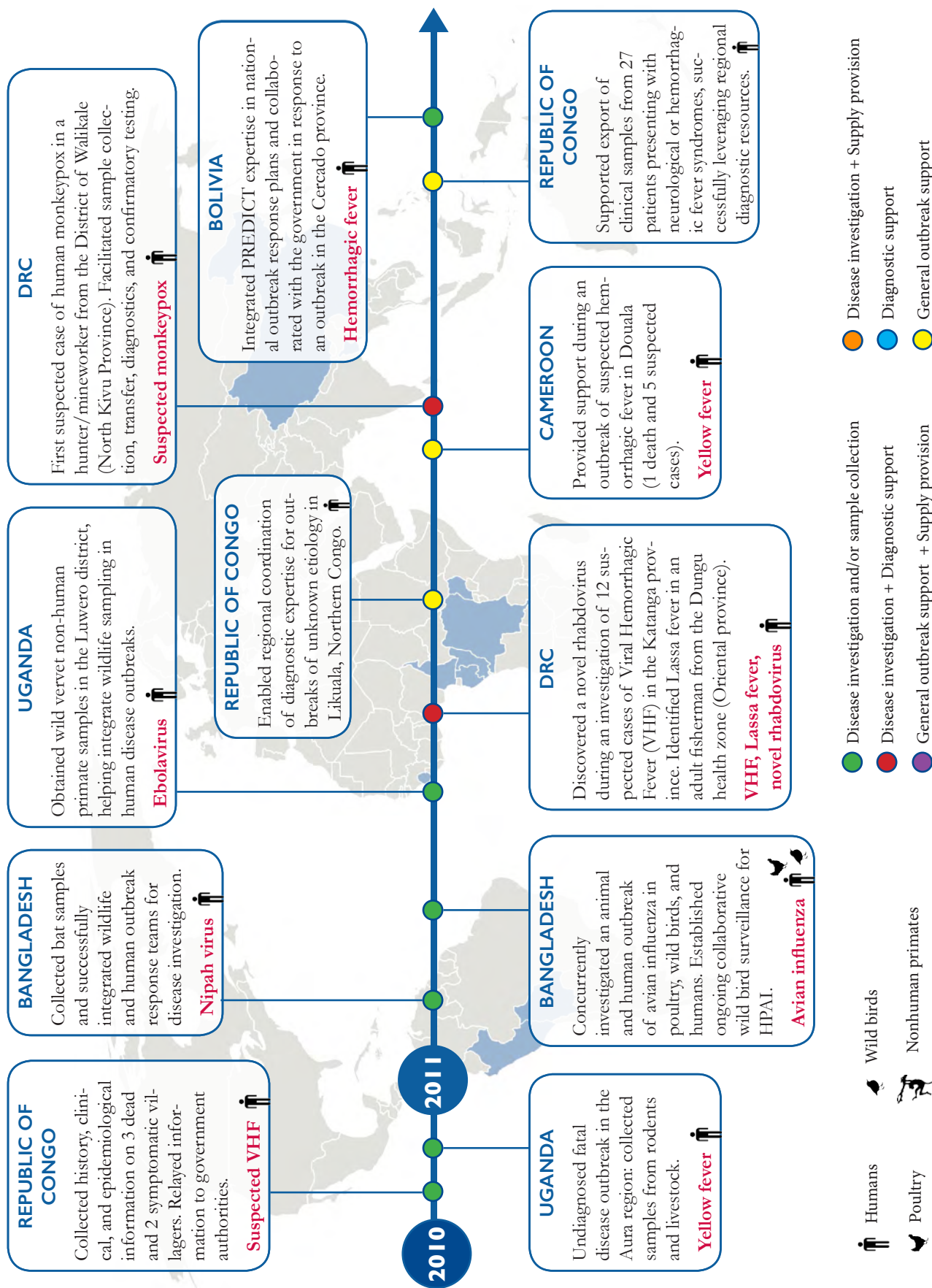
The *Outbreak Response Timeline* describes the support provided during outbreak response efforts. Additional information on outbreak response efforts is included in the country-specific sections where relevant.

PREDICT OUTBREAK RESPONSE

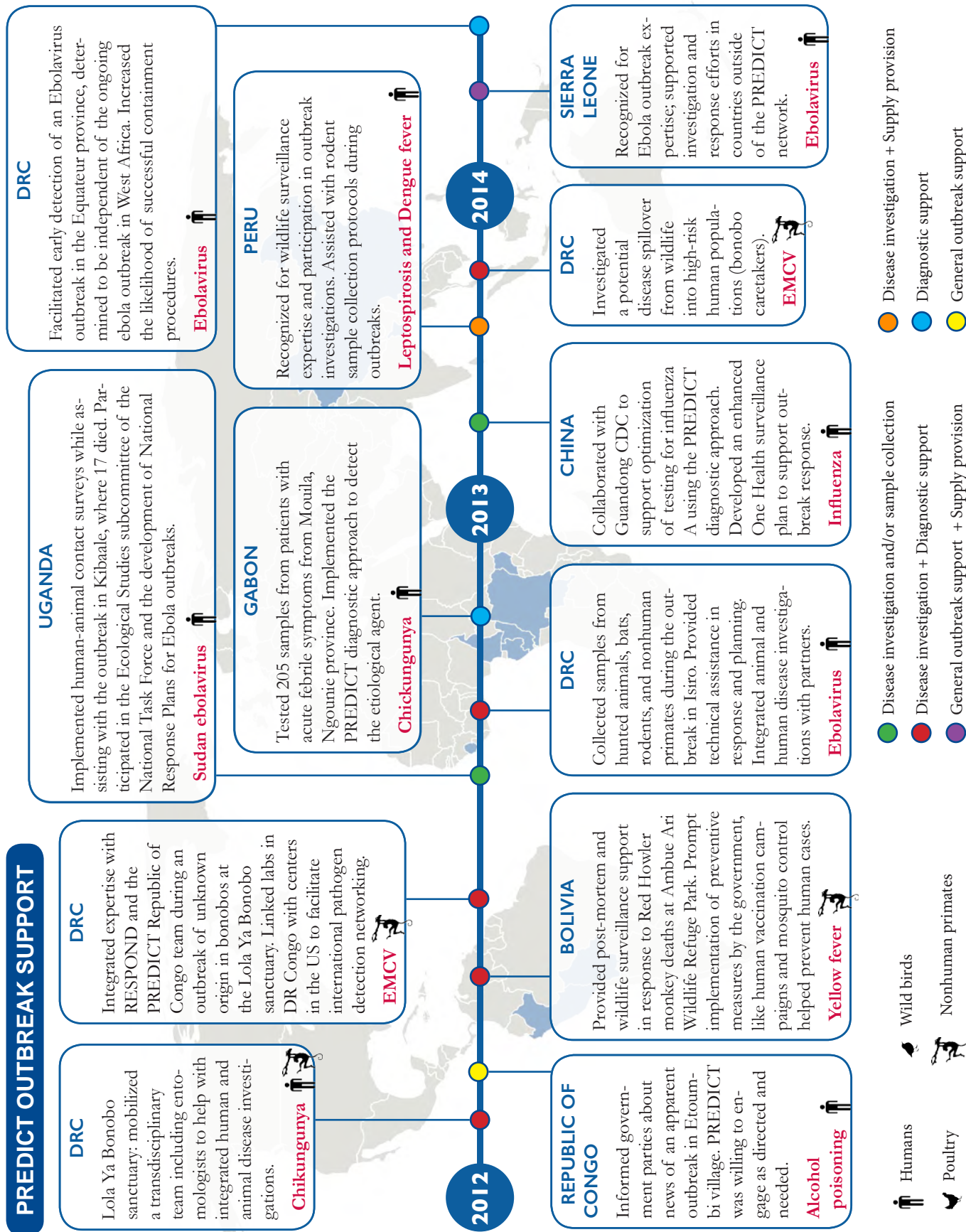
23 outbreaks in 10 countries



PREDICT OUTBREAK SUPPORT




PREDICT OUTBREAK SUPPORT



"All support was conducted at the request of government authorities and in coordination with EPT partners, including other international health response organizations"

OPERATIONALIZING ONE HEALTH





Recent examples of emerging zoonoses, such as H1N1 pandemic influenza, H5N1 and H7N9 avian influenza, Ebola virus disease, SARS, and MERS, serve as a reminder that the health of humans, animals, and ecosystems are interconnected and that early detection and response to emerging zoonotic pathogens requires a coordinated, interdisciplinary, collaborative, and cross-sectoral approach at local, regional, and global levels.

In addition to the burden on human and animal health, the economic impacts associated with emerging and pandemic pathogens can be catastrophic, including costs associated with decreased commerce, travel, and tourism as well as those incurred from treatment and control efforts (Karesh et al. 2012). The One Health approach – an interdisciplinary collaborative effort to attain optimal health for people, animals, and our environment (Figure 1) – has gained popularity since the emergence of SARS and H5N1 highly pathogenic avian influenza A in Asia, which demonstrated the critical need for a coordinated interdisciplinary strategy for disease recognition, prevention, and control (World Bank 2012). As our world becomes increasingly connected through global trade and travel, emerging diseases pose a greater threat to the global community, requiring collaboration between ministries of health and institutions involved in health, trade, agriculture, and the environment (Karesh et al. 2012). The One Health approach presents important opportunities to reduce the impact of emergence events and also to prevent future emergence through improved knowledge and coordination.

Despite increasing endorsement of One Health by agency officials and policy makers globally, the lack of cross-sectoral and transboundary collaboration coupled with siloed resources have limited widespread implementation of the approach (Murray and Aviso 2011). However, with the growing international support for One Health, as illustrated by a recent tripartite organization (World Health Organization (WHO), Food and Agriculture Organization (FAO), and World Organization for Animal Health (OIE)) joint action plan to address health risks at the human-animal-ecosystem interface (FAO, OIE, WHO 2010), the value of an integrated approach to global surveillance and disease control and prevention is increasingly being realized.

One Health in Action

Through integrated efforts to promote health, effective natural resource management, and development, the PREDICT project, along with other partners under USAID's EPT program, have advanced One Health capacity and infrastructure in over 20 countries considered hotspots for zoonotic disease emergence. PREDICT worked cooperatively with a wide range of government ministries, scientific institutions, local organizations, and other stakeholders to further local and global One Health initiatives by enabling a structure for effective collaboration across disciplines and geographic boundaries. Utilizing a One Health approach, PREDICT

focused its efforts on strengthening surveillance and diagnostic laboratory capacity to detect known zoonotic and novel pathogens at the wildlife-human interface, monitoring human-wildlife interactions for the potential spillover of pathogens posing a health threat (Figure 2), and investigating ecological drivers of pathogen spillover into people with the ultimate aim of improved prevention of zoonotic disease emergence. By emphasizing the links among human, animal, and environmental health, PREDICT enabled and supported integrated efforts to promote public health, effective natural resource management, and development. Examples include:

- **Training:** PREDICT and its partners trained 2,522 local individuals, including 842 women, on biosafety, surveillance, laboratory techniques, and outbreak investigation. Participants included government officials, physicians, veterinarians, resource managers, laboratory technicians, hunters, and students. Training in One Health approaches were also implemented during disease outbreak response and investigations, such as for Ebola virus disease in Uganda and DRC, influenza A virus in China, and Nipah virus outbreaks in Bangladesh.

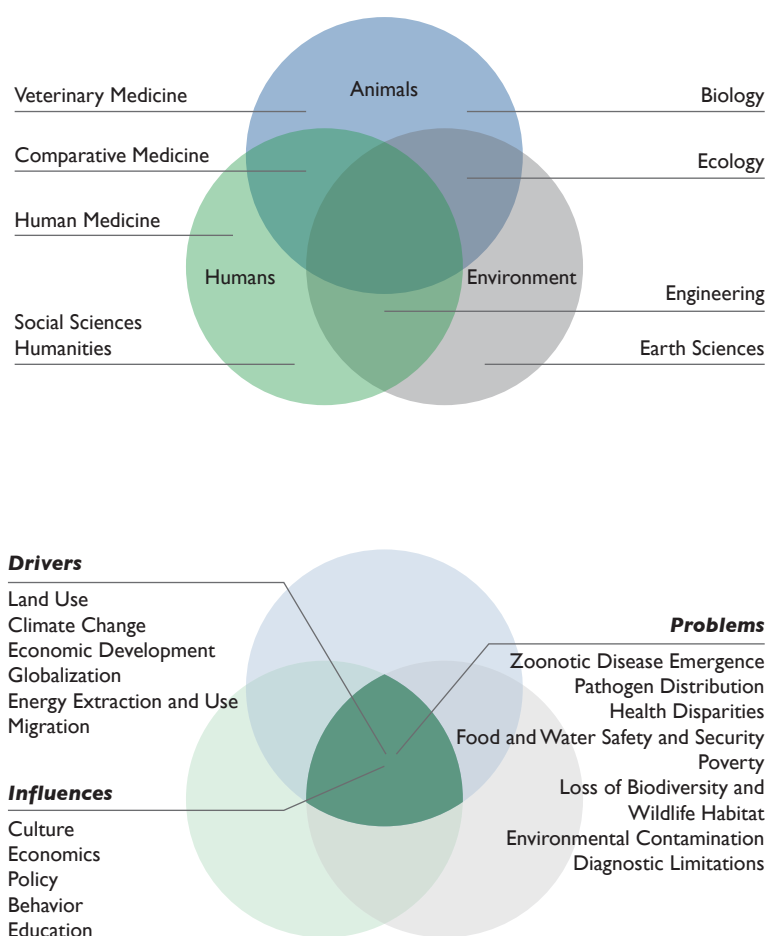


Figure 1. The One Health concept recognizes the interrelationship between animal, human, and environmental health (top) and promotes interdisciplinary collaborations to solve complex health problems (bottom). Adapted from Mazet et al. in press.

- Integrated Approaches:** PREDICT countries developed mechanisms for overcoming geographic and disciplinary constraints. Multidisciplinary collaboration enabled targeted and sustainable advancements, including sharing and interpretation of surveillance results across ministries, as well as partner-driven implementation of PREDICT surveillance and testing protocols. Partners frequently participated in or co-organized PREDICT surveillance and diagnostic efforts, and PREDICT team members were invited to serve on national task forces and to provide technical assistance for disease response and prevention efforts.

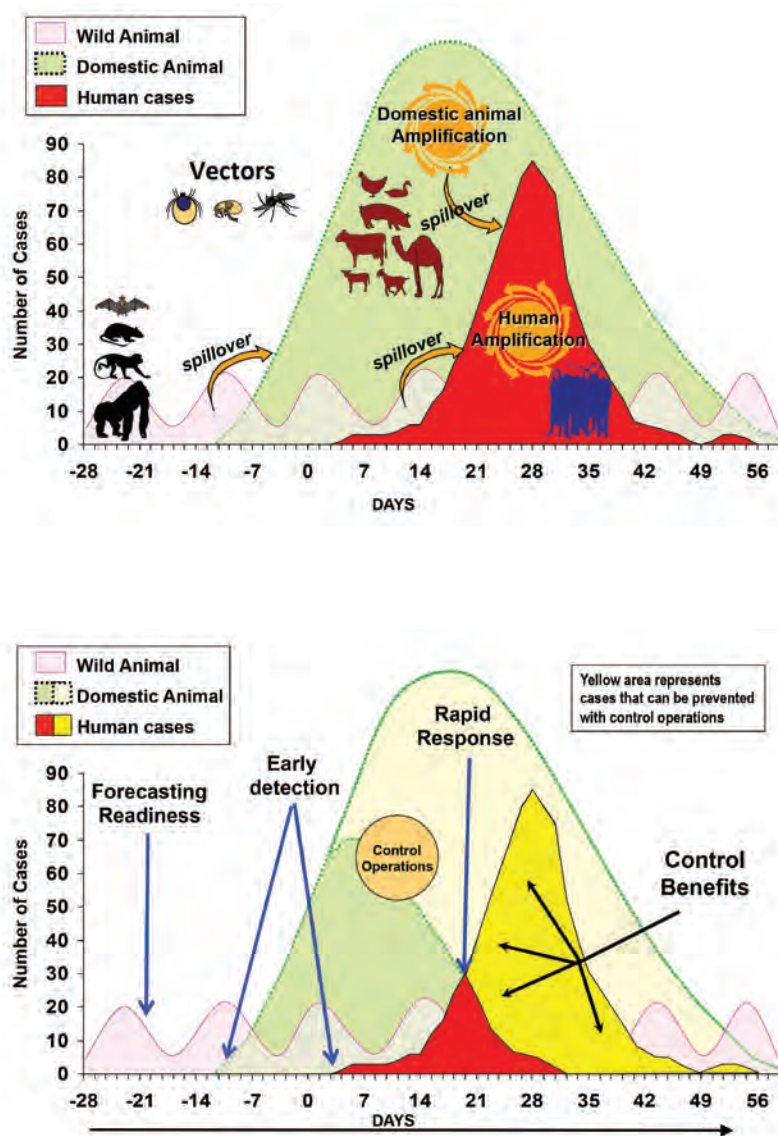


Figure 2. (Top) Transmission to and amplification of zoonotic pathogens in people (red) occurs after a pathogen from wild animals (pink) spills over into livestock (green) or people to cause an outbreak. Spillover arrows illustrate cross-species transmission.

(Bottom) Forecasting and early detection and control efforts reduce disease incidence in animals (green) and people (red). Adapted from Karesh et al. 2012.

- **Stakeholder Engagement and Leadership:** In addition to engaging public health, environment, and agricultural ministries, PREDICT facilitated strong partnerships among other key stakeholders. The many partnerships include collaboration with development boards, extractive industries, wildlife farms, indigenous leaders, hospitals, hunters, and rural communities.

In addition to directly supporting local One Health advancement in EPT countries, PREDICT protocols, approaches, and scientific findings provided guidance for intergovernmental agencies, the research community, and other countries. PREDICT fostered regional collaboration and eagerly provided expertise and best practices as invited by international stakeholders. PREDICT outputs have enabled implementation of effective One Health strategies globally. Examples include:

- **Expertise Networks:** PREDICT enabled the efficient functioning of the IUCN Species Survival Commission Wildlife Health Specialist Group, a global network of over 300 multidisciplinary experts that serves as a first response to wildlife health concerns around the world. Through leadership of the OIE Working Group on Wildlife Diseases, and participation in regulation-setting sessions, PREDICT provided guidance on the role of wildlife in diseases of high priority to international trade and zoonotic disease emergence.
- **Technical Guidance:** PREDICT partners were frequently called upon to provide technical input on intergovernmental One Health strategies, including informing approaches for optimizing influenza A virus surveillance in wild birds (as requested by the FAO/OIE's OFFLU), serving as an expert on the human-animal interface for the World Health Organization International Health Regulations, and collaborating with the Convention on Biological Diversity on health considerations related to biodiversity. PREDICT protocols were utilized for outbreak response and preparedness efforts outside of PREDICT countries, including in the Middle East Respiratory Syndrome (MERS-CoV), H7N9, and Ebola virus disease in Sierra Leone.
- **Capacity Building:** PREDICT widely supported the development of professionals engaged in One Health activities, enabling participation of both PREDICT team members and in-country partners at global scientific meetings, including the OIE Global Conference on Wildlife-Animal Health and Biodiversity and the International Congress on Pathogens at the Human Animal Interface. PREDICT supported WildHealthNet (WHN), an online, peer-to-peer African network being developed with African Union-InterAfrican Bureau for Animal Resources (AU-IBAR). In 2013 alone, WHN membership grew to over 130 members (representing 23 African nations), with approximately one third of these members being women.
- **Dissemination of Information:** To ensure information was widely disseminated and can be implemented beyond PREDICT countries, PREDICT partners presented findings and approaches at scientific meetings and published over 90 peer-reviewed papers (see http://www.vetmed.ucdavis.edu/ohi/predict/predict_publications.cfm). In addition, PREDICT sampling data and test results were posted on the HealthMap interface (see <http://www.healthmap.org/predict/>) to feed into global surveillance and remote sensing systems. PREDICT partners frequently provided key findings to major health related bodies and organizations such as WHO, FAO, OIE, the US Institute of Medicine, US Department of Homeland Security, US National Institutes of Health, and the US Centers for Disease Control and Prevention.

Future Opportunities for PREDICT Countries to Build on One Health Advancements:

- Creating national strategies for the sustainability and broadening of One Health initiatives, including systems for disease prevention and early detection;
- Engaging and training additional stakeholders, including representatives from additional ministries, health and industry sectors, and local at-risk communities;
- Documenting One Health approach success stories for proof of concept; and
- Boosting regional collaboration on pandemic prevention and preparedness.

Through its local and global One Health initiatives, PREDICT and its partners enabled a structure for effective collaboration across disciplines and geographic boundaries to promote global health through prevention of emerging zoonotic disease threats. This infrastructure can be built upon to include additional regions and stakeholders for further impact.

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INFLUENZA



When called upon by host governments, PREDICT provided assistance with influenza outbreak response and preparedness by providing technical guidance and transfer of technologies. PREDICT also worked to address critical research gaps as needed to inform on global surveillance and testing strategies by examining the factors influencing the emergence and spread of influenza A viruses, especially from or by potential wildlife reservoirs.

While PREDICT surveillance activities were not initially designed to specifically target influenza A viruses, our diagnostic strategy did include protocols to detect the viruses. Thus PREDICT contributed protocols for testing to laboratories as needed and also facilitated influenza A screening of wildlife and selected human samples in collaborating laboratories.

INFLUENZA A H7N9 VIRUS OUTBREAK INVESTIGATION

In 2013, an H7N9 influenza A virus strain caused a deadly outbreak in people in the provinces of southeastern China. This outbreak came as a surprise, as this strain had never before been diagnosed as the cause of disease in people. PREDICT researchers investigated potential source populations and the conditions for the genesis of the H7N9 virus outbreak using active surveillance, screening of virus archives, and evolutionary analyses. This research revealed that the H7N9 outbreak lineage originated from reassortment of H7 viruses and enzootic H9N2 viruses and that the H7 viruses likely were transmitted from domestic ducks to chickens in China during two separate events (Lam et al. 2013). The researchers discovered a related H7N7 influenza virus in chickens that has the ability to infect mammals experimentally, which provides evidence that H7 viruses may pose a greater public health threat than previously recognized (Lam et al. 2013).

Also, in response to the knowledge gaps surrounding the source of infection for human cases of the avian H7N9 subtype in China, PREDICT estimated the historical prevalence and distribution of H7N9 viruses in wild bird populations. Because the prevalence of the H7N9 subtype was found to be historically low in wild birds, the researchers recommended that future work should focus on identifying H7N9 sequences

Live animal markets in southern China selling a mix of wild birds, domestic poultry, and mammals.



PHOTO BY JON EPSTEIN

that are linked to increased human pathogenicity and transmissibility and to conduct risk-based surveillance to detect these viruses in domestic and wild birds (Olson et al. 2013).

Genetic analysis has provided evidence that the human H7N9 viruses in this outbreak were of avian origin through novel reassortment of the influenza A virus subtypes H9N2 and H7N3 (Gao et al. 2013). PREDICT was the first to report H9N2 viruses in wild birds and suggested that wild birds were natural reservoirs and may carry the virus along migratory routes allowing for transmission to other host species (Zhu et al. 2013). Fifteen H9N2 viruses were isolated from two species of wild ducks (spot billed ducks and mallard ducks) in Poyang Lake of southeast China in 2011. Eleven representative viruses were further characterized by complete sequencing of the eight gene segments. One isolate replicated efficiently in laboratory mice tissues and led to mortality in 20-40% of experimentally infected mice cohorts (Zhu et al. 2013) revealing the virus's ability to cause fatal infections in a mammalian species and therefore, the potential to pose a threat to human health.

SURVEILLANCE STRATEGIES FOR INFLUENZA A VIRUSES

In an effort to improve surveillance and detection of new influenza subtypes and outbreaks, scientists are looking to better understand and monitor the diversity, ability to change genetically, and distribution of all influenza A viruses – not just those known to cause disease. By completing the first global inventory of influenza A strains in birds and mammals, PREDICT provided a key step in building that understanding and providing new insight into competent bird and mammal hosts and drivers of viral diversity and the emergence of influenza in domestic animals and people.

In order to understand the role wild birds play in the emergence of zoonotic influenza viruses, PREDICT scientists examined 11,870 sequences from the GenBank database to provide a baseline inventory and insight into patterns of global influenza A subtype diversity and richness in wild birds. Further, they conducted an extensive literature review and communicated directly with scientists to gather data from 50 studies and over 250,000 birds to assess the historic sampling effort to better understand the current knowledge. Virus subtype richness was examined in order to estimate the diversity of influenza subtypes in a particular location using sample-based accumulation curves that examine the presence and absence of a subtype in each location.

This research identified over 116 influenza A strains in wild birds globally, which is approximately twice the number found in domestic birds. The majority of all known sequenced subtypes were found in wild ducks, geese, and swans (Anseriformes; 90%) or gulls and shorebirds (Charadriiformes; 63%); mallard ducks were found to carry the highest number of viral strains (Olson et al. 2014). The more a strain was shared across wild bird types, the more likely it was to be found in domestic birds, and thus thought to be a risk factor for spillover events. Geographically, the North American Atlantic flyway, Europe, and Asia were global hotspots for subtype richness (Olson et al. 2014). Results from the analysis showed that sampling plans for surveillance needed to include a minimum of 10,000 samples to detect an estimated 75% of circulating virus subtypes from a targeted bird population, and thus should help to guide future surveillance to understand the influence of host and virus biodiversity on emergence and transmission.

In an effort to understand factors driving the evolution and diversity of all high-risk influenza A virus subtypes and more accurately identify hotspot areas of emergence to better design diagnostic strategies, PREDICT investigators also evaluated mutation rates of high priority influenza A subtypes detected globally as well as socio-economic, biodiversity, and agricultural drivers that may be associated with subtype diversity and reassortment. Results indicated that potentially pathogenic influenza A strains may be more likely to evolve in East Asia, reinforced by the fact that the majority of subtypes that have caused disease and mortality in humans in recent years, such as H5N1, H5N6, H6N1, H7N9, H9N2 and H10N8, were first detected in China and Hong Kong. Other factors that were associated with detected subtype diversity were sampling effort, measured by the number of strains reported, and healthcare spending, as a measure of the ability to test and detect multiple influenza subtypes. An important recommendation for diagnostic testing was to revise current strategies of targeted surveillance for specific influenza subtypes – instead performing broader testing to detect all subtypes in order to better understand the total diversity globally and to facilitate the early detection of emerging subtypes and strains.

FACTORS DRIVING EMERGENCE AND PERSISTENCE OF H5N1 INFLUENZA A VIRUSES

PREDICT also examined drivers for highly pathogenic H5N1 influenza A persistence. Hosseini et al. (2013) used mathematical models of H5N1 virus dynamics in different-sized poultry farms to understand the virus' ability to persist in different types of poultry operations and to investigate the effects of culling and cleaning as control measures. Results indicated that moderately-sized poultry farms can sustain H5N1 virus for over two years without wild bird involvement. In addition, a mixture of intensive and backyard farming within a country sustained H5N1 infection and circulation indefinitely (Hosseini et al. 2013). In countries with a need for intensive poultry production, larger scale commercial poultry operations with more intensive H5N1 virus monitoring and increased biosecurity may be the best strategy for reducing risk of human infection with H5N1 virus and persistence in farmed poultry (Hosseini et al. 2013).

In addition, Murray and Morse (2011) assessed whether human H5N1 cases occurred seasonally in Indonesia and Egypt in association with changes in temperature, precipitation, and humidity. The incidence of human H5N1 in Egypt, but not Indonesia, was strongly associated with meteorological variables. In addition, incidence of infection was highest in Egypt when precipitation was low, and temperature, along with absolute and relative humidity, were moderate compared to the average daily conditions in Egypt; suggesting that human infection may be occurring primarily via droplet transmission from close contact with infected poultry (Murray and Morse 2011).

A vendor sells poultry by the roadside in Guangdong, China.



PHOTO BY JON EPSTEIN

CONTRIBUTIONS TO INFLUENZA RESEARCH IN OTHER WILDLIFE SPECIES OF INTEREST: EMERGENCE OF INFLUENZA A STRAINS IN MARINE MAMMALS

Transmission between wild avian reservoirs and mammalian hosts is an important factor in the dynamics and evolution of influenza A viruses. Marine mammals come into contact with aquatic birds presenting opportunities for interspecies transmission and the emergence of new strains of influenza viruses that may pose a risk to public health. Previous studies have documented interspecies transmission events between marine mammals and birds and marine mammals and people (Webster et al. 1981; Hinshaw et al. 1984; Mandler et al. 1990).

In 2009, a new strain of H1N1 emerged in people and resulted in a global pandemic. The following year, PREDICT investigators detected pandemic H1N1 influenza in free-ranging northern elephant seals in 2010 off the central California coast upon their return from their migration. Virus isolation, whole genome sequencing, and hemagglutination inhibition assay confirmed exposure to pandemic H1N1 influenza virus in the seals. In vitro characterizations showed that replication of the virus was similar to that of reference strains of pandemic H1N1 in canine kidney cells. However, the virus did not replicate well in human epithelial respiratory cells, demonstrating that the virus isolates may be elephant seal adapted (Goldstein et al. 2013). This was the first isolation of H1N1 in a marine mammal. These findings provided evidence for cross species transmission of influenza viruses among free-ranging wildlife and between wildlife and people and provided evidence that oceanic transmission and movement of pathogens should not be eliminated from consideration of amplification and spread (Figure 1; Goldstein et al. 2013).

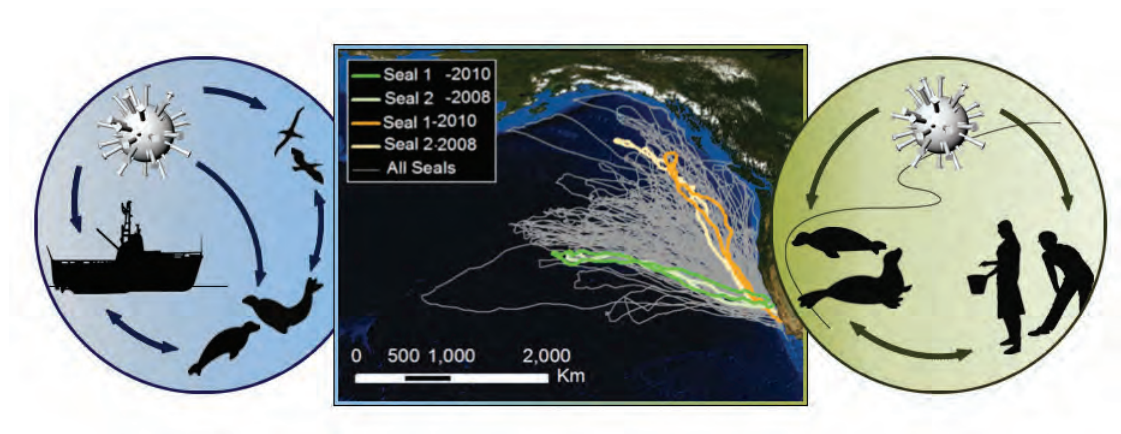


Figure 1. A visual representation of the geographical and interspecies spread of influenza A. In 2010, exposure to H1N1 was documented in free-ranging northern elephant seals after returning to the California coast from their short migration in the northeast Pacific. Given that direct contact between people and elephant seals is unlikely while at sea, exposure may have occurred through contact with aquatic birds, thus expanding the virus's host and geographical range by circulating among marine mammals, birds, and people on land and at sea. From Mazet et al. (in press).

The following year, in 2011, 162 harbor seals died off the New England coast of the US in an outbreak of pneumonia. A PREDICT investigator detected an influenza A virus H3N8 subtype in the harbor seals that died, a subtype typically associated with infection of avian, equine, and canine hosts. Sequence analysis revealed that the H3N8 influenza A subtype was most similar to avian H3N8 strains previously detected in North American waterfowl, but with mutations consistent with adaptation to mammalian hosts (Anthony et al. 2012).

Both of these examples documented the emergence of new strains of influenza viruses in new mammalian hosts and are of public health concern because of the potential for viral persistence and cross-species transmission. In both cases, the viruses had naturally acquired mutations that may increase transmissibility and virulence in mammals. Monitoring the spillover and adaptation of avian viruses in mammalian species is critically important if we are to understand the factors that lead to both epizootic and zoonotic emergence.

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DEEP FOREST



PREDICT implemented the Deep Forest (DF) study to enhance the understanding of ecological factors that drive zoonotic disease emergence due to land-use change. It has and will continue to refine our approach in the global scale “hotspots” modeling, by providing detailed information about risk at a local level – a scale at which humans live and interact with wildlife and livestock.

Characterizing known and unknown viral diversity and describing the relationship between viral diversity, host diversity, land-use change, and human ecology is critical for better understanding of the ecological processes behind zoonotic disease emergence so that disease outbreaks can be prevented.

Approximately 20% of novel emerging infectious diseases (EIDs) and 50% of emerging and re-emerging zoonotic diseases have been attributed to land-use change. Land-use changes are thought to affect the risk of cross-species transmission (“spillover”) by perturbing the dynamics of pathogens in wildlife hosts and/or by bringing novel host-pathogen pairs (including humans) into contact for the first time (Murray and Daszak 2013).

The Deep Forest study aimed to evaluate how increasing land development influences 1) patterns of biodiversity; 2) corresponding patterns of viral diversity; and 3) patterns of human occupancy, abundance, and behavior that may influence contact rates with wildlife in changing landscapes.

The study spanned three continents – South America (Brazil), Asia (Sabah in Malaysian Borneo), and Africa (Uganda). Each country presents an excellent opportunity as a model system because each is highly biodiverse and under extensive pressure from land-use changes, factors that render them among the world’s “hotspots” of disease emergence risk.

Deep Forest employed a systematic, gradient-based sampling scheme. Each country where DF was conducted contained nine field sites – three sites in each of three land-use gradient levels: pristine (low disturbance), intermediate (medium disturbance), and disturbed (high disturbance). Landscape disturbance was measured at two scales: 1) the landscape scale, calculated from satellite imagery, and 2) the local (site) scale, calculated from on-the-ground surveys. Deep Forest biodiversity measurements focused on the three high-risk taxonomic groups targeted for PREDICT: rodents, bats, and nonhuman primates. At each DF site, standardized wildlife surveys were used to characterize local species richness and diversity. From each animal captured within these surveys, blood, saliva, and rectal swab samples were obtained. Urine and feces were also opportunistically collected.

Samples are currently being analyzed in the laboratory by consensus PCR for the detection of known and new viruses. A subset of the samples has also been prepared for metagenomic deep sequencing, which allows for the detection of the entire community of viruses within the

samples. Field sampling was conducted over a period of two years, with each site sampled twice, once during each season, to minimize the effect that seasonality might have on the likelihood of detection of both host and viral species.

In addition to viral surveillance, biodiversity surveys, and landscape disturbance analysis, the Deep Forest Human Contact (DFHC) survey was implemented to characterize human-animal contact at the landscape scale. At each site, particular attention was paid to contact with bats, rodents, and primates, as well as other types of wild and domestic animals to which people are frequently exposed. While the core questions asked were the same across all three countries, the survey was adapted based on results from qualitative focal group research, then tailored to the country, local population, and setting in which it was implemented. Results from the surveys indicate how human-animal contact, a fundamental but poorly quantified measure in disease systems, varies with land-use practices and intensity of disturbance.

FRAMEWORK AND KEY RESULTS

PREDICT developed a framework of sampling and analysis (building off Lloyd-Smith et al. 2009) for the three focal areas of Deep Forest, representing three key components of spillover potential (pathogen, contact, and transmission potentials; see Figure 1 below), for an unknown pathogen with wildlife origins. Figure 1 also shows how both survey methodologies allowed us to capture each parameter critical for our analysis of disease emergence risk: 1) wildlife and viral survey (blue box) and 2) human behavioral survey (DFHC) (green boxes), both undertaken across a land-use development gradient (brown shading), which represents the key driver (land-use change) of disease emergence under investigation in the DF study (see also Hotspots II in the Modeling and Analytics section for a global level analysis of the effect of land-use change on disease emergence).

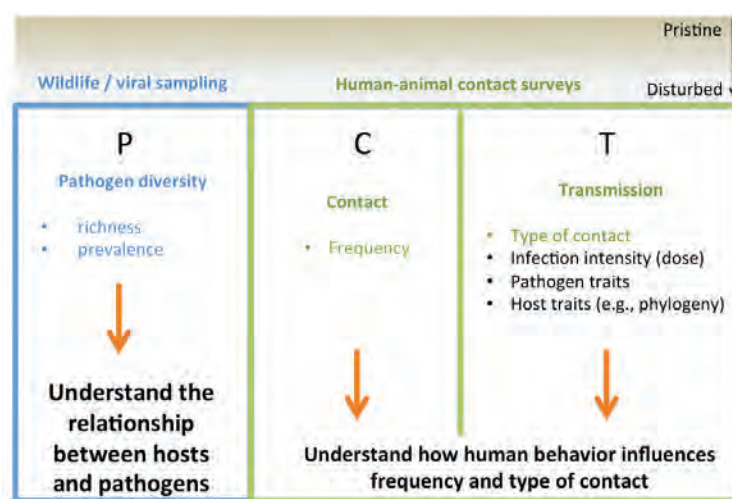


Figure 1. Schematic of Deep Forest survey components and sampling design. Descriptions of each box appear in the text above and below. P = pathogen potential; C = contact potential; T = transmission potential.

Wildlife and Viral Sampling

Pathogen potential (P in Figure 1) represents the pathogen pool and is investigated in DF via viral diversity (richness/abundance relationship), that is the diversity of viruses available for transmission in a landscape, such that more viral diversity or greater viral prevalence in a landscape likely represents greater risk of spillover. Viral diversity is necessarily seen through the filter of wildlife hosts, so understanding the relationship between host and viral communities

will allow us to probe the ‘depth’ of the pathogen pool (blue box in Figure 1) from which novel pathogens may emerge.

In total, PREDICT sampled 2,136 animals across the three continents as part of the DF study. Within just Malaysia, we have tested 2,546 samples from 1,001 animals and preliminarily identified 165 samples from 72 animals that were positive for viruses by testing in-country for up to 17 viral families and by testing at the Center for Infection and Immunity at Columbia University for four viral families, demonstrating that both known and many unknown viruses will be detected along the land-use change gradients. Preliminary results from an additional 2,730 specimens obtained from another 901 animals in the three countries, tested so far for just four viral families, have already yielded another 40 samples that were positive for viruses. Analyses of these data will follow completion of testing for all samples for remaining viral families.

Deep Forest Human Contact (DFHC) Survey

Contact potential (C in Figure 1) is the capacity for a landscape to sustain spillover-relevant contact ‘events’ (e.g. eating or encountering wildlife) and is dominated by **contact frequency** (where more events in a landscape is hypothesized to represent greater risk of spillover; green box in Figure 1). The DFHC surveys asked respondents carefully-designed questions from which an estimate of contact frequency (e.g. sometimes vs. never) for numerous different contact types (see Transmission potential below) could be inferred. These frequencies were then multiplied by the population size that occurs across the landscape in order to give a relative indication of the landscape level contact rate for each contact type. Results from DFHC analyses are presented below.

Transmission potential (T in Figure 1) is the capacity for a landscape to promote factors that increase the likelihood of transmission given a contact event. Although numerous factors may influence likelihood of transmission given contact, we focused on the **type of contact** event (more ‘risky’ contact types are hypothesized to represent greater risk of spillover), where riskiness is assessed primarily via expert opinion, taking into consideration additional factors, such as duration or ‘intimacy’ of contact (e.g. eating vs. seeing). The DFHC surveys asked respondents about a range of different direct and indirect contact types that represent potential risk factors for disease spillover. Examples include wildlife hunting, butchering, or consumption and seeing wildlife or their fecal material in the home. For analyses, the *contact potential* (see above) is combined with *transmission potential* to map landscape-level relative risk for each contact type.

PREDICT collected more than 1,200 standardized DFHC surveys across the Deep Forest sites. Figure 2 shows the distribution of sample sizes for each gradient level.

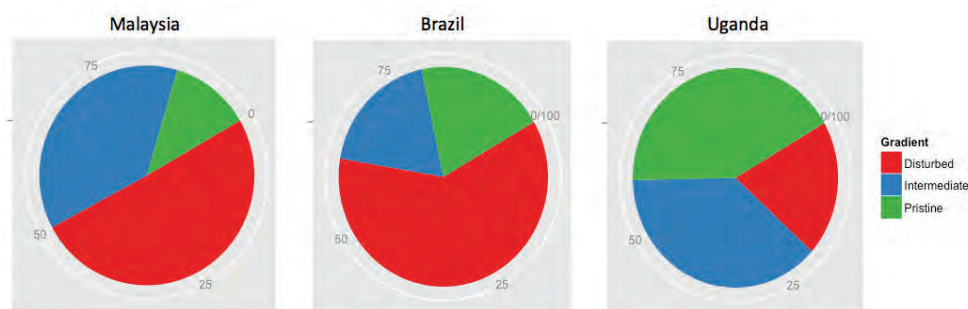


Figure 2. DFHC survey sample sizes and proportions across three countries (and continents) and gradient levels.

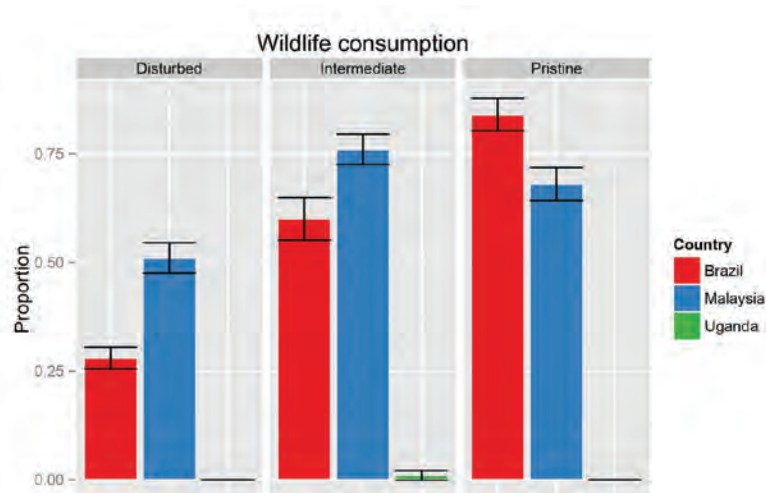


Figure 3. Proportion of respondents reporting wildlife consumption across three countries (and continents) and three gradient levels. Error bars at 95% confidence intervals around the estimated proportion of respondents reporting wildlife consumption.

Figure 3 illustrates the effect of the gradient level on one high-risk behavior, the **consumption of wildlife**, just one example of a specific *contact type* (direct contact) of interest. Self-reported wildlife consumption patterns differed considerably among countries and gradient levels and may be partially reflective of cultural differences and laws and their enforcement regarding wildlife protection. Generally speaking, there was less wildlife consumption reported in Uganda than in Brazil or Malaysia, and less wildlife consumption in the disturbed sites compared to the intermediate and pristine sites in the two countries reporting high wildlife consumption (Brazil and Malaysia). This finding has implications for the risk of spillover from wildlife as a result of wildlife

consumption in different cultural contexts and illustrates why all potentially high-risk behaviors should be explored across geographic and cultural zones.

Mapping Human-animal Contact

In order to understand how human-animal contact relates to landscape-level risk of disease spillover, we also took into account the spatial distribution of both landscape disturbance and the human population size that inhabits our study landscapes. Methodologically, we developed a four-step process (Figure 4, below). The results show that even though wildlife consumption is generally higher in pristine areas (see above), the overall contact rate with animals due to consumption does not follow the same pattern, with population size driving the overall rate of human animal contact at the landscape level within each country. Importantly, the results show that wildlife consumption based human-animal contact varies spatially both within and across countries (not different scales used between countries).

CONCLUSION AND NEXT STEPS

In DF, we hypothesized that landscapes with more viruses available (deeper viral pool) contribute to greater spillover risk, holding the other risk factors constant (contact types and contact rates – see Figure 4). We will empirically relate the depth of the viral pool (from which novel pathogens may emerge) to wildlife diversity and abundance, with final analysis and results pending completion of viral testing. Once available for analysis, our wildlife and viral results will be combined with the models presented above to estimate the relative risk of novel viral spillover due to land-use change. While the aim of the DF study is to estimate the relative risk of spillover of *unknown* pathogens from wildlife in dynamic landscapes, the approach could also be used to help understand the risk of spillover for known pathogens. For example, because human-animal contact is a risk factor for many known diseases (e.g. Ebola virus disease), quantifying bushmeat consumption and contact rates at the landscape scale, as shown in Figure 4, could also help focus surveillance or mitigation activities to reduce the risks of pathogen spillover from bushmeat consumption. The same systematic approach could be applied to better identify the risks attributable to other hypothesized drivers of disease emergence.

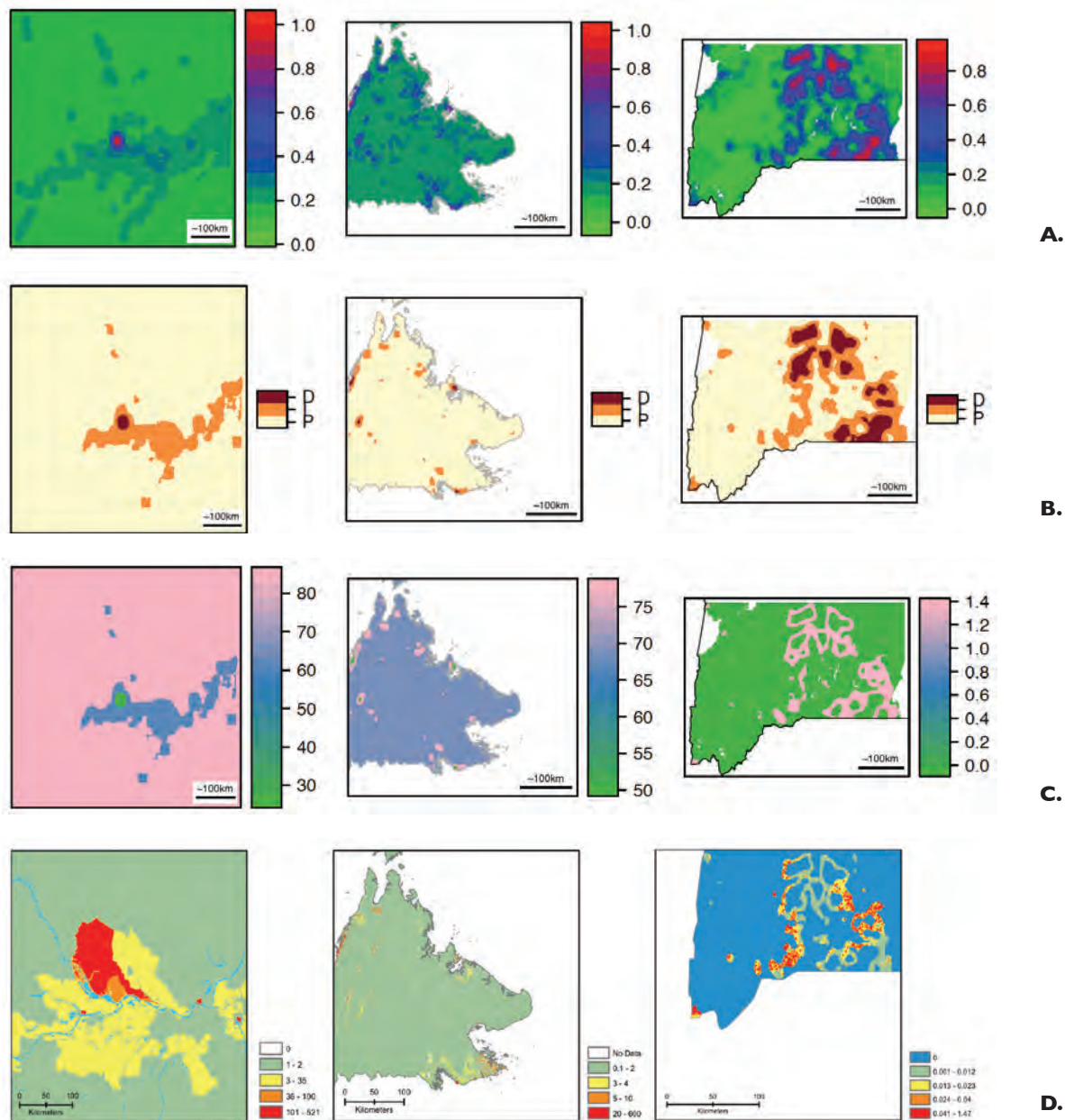


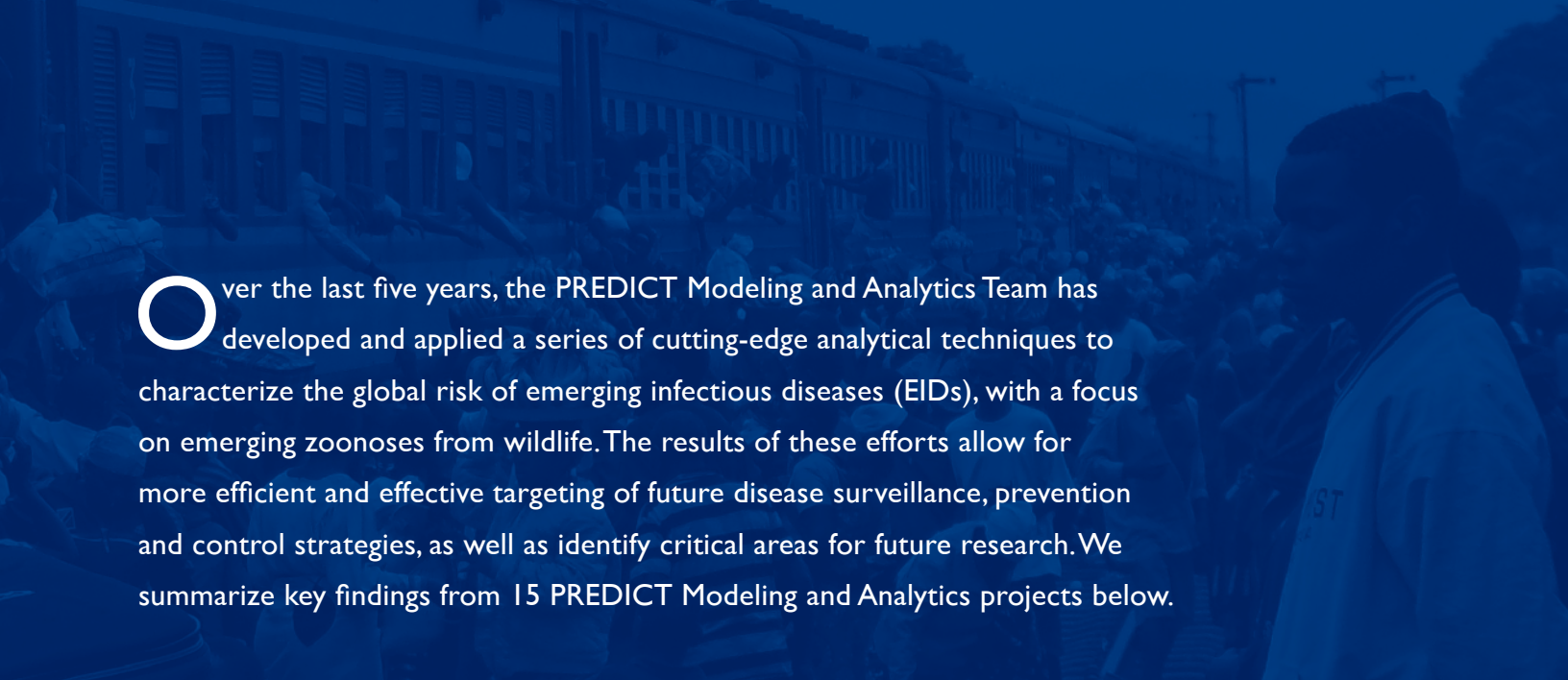
Figure 4. Steps showing the creation of relative human-animal contact rate maps at the landscape level for Brazil (Left Column), Malaysia (Middle Column), and Uganda (Right Column). The contact type illustrated here is wildlife consumption as reported in the DFHC survey. A) Step 1: Raw landscape disturbance index (LDI) calculations for each country (range 0 = pristine, 1 = highly disturbed). B) Step 2: Reclassified LDI to match disturbance gradient levels used for wildlife and viral sampling and DFHC surveys (P = pristine, I = intermediate, D = disturbed). C) Step 3: Percentage of respondents reporting wildlife consumption mapped to gradient scale. D) Step 4: Final result illustrating relative human-animal contact rate (scale is an index of consumption contacts per grid cell) at the landscape scale, derived by multiplying the proportion of respondents reporting wildlife consumption by human population size per pixel.

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MODELING AND ANALYTICS





Over the last five years, the PREDICT Modeling and Analytics Team has developed and applied a series of cutting-edge analytical techniques to characterize the global risk of emerging infectious diseases (EIDs), with a focus on emerging zoonoses from wildlife. The results of these efforts allow for more efficient and effective targeting of future disease surveillance, prevention and control strategies, as well as identify critical areas for future research. We summarize key findings from 15 PREDICT Modeling and Analytics projects below.

These include:

- **Hotspots II:** An updated model of the risk of novel disease emergence from wildlife
- **High-Risk Interfaces for Disease Emergence and Spread**
- **Sicki GRID:** Online global repository of disease emergence events and information
- **Climate Change and EID risk:** Case study of Henipaviruses in bats
- **Host-Pathogen Phylogeny Project:** Host and viral traits to predict zoonoses
- **Virodiversity:** Estimating the number of undiscovered viruses in mammals
- **Targeting viral discovery in symptomatic vs. asymptomatic animals**
- **Viral Survival:** Analysis of data for how long viruses live in the environment
- **Transmission Pathways:** Targeting zoonoses surveillance and control
- **What-If Scenarios:** Potential to stop Virus X from becoming a pandemic
- **Travel and Trade:** Global vulnerability to EID emergence and spread
- **Strategy to Rank Zoonotic Potential of Novel Viruses**
- **Economic Analysis of Pandemic Mitigation vs. Adaptation**
- **Quantifying Economic Impacts of EIDs:** Case study of SARS
- **Demographic Conditions for Outbreaks:** Case study of Ebola in West Africa

HOTSPOTS II: AN UPDATED MODEL OF THE RISK OF NOVEL DISEASE EMERGENCE FROM WILDLIFE

The incidence of EID events has increased over the past several decades. To improve our understanding of the underlying causes for emergence, PREDICT built upon previous research to assess temporal and spatial patterns of disease emergence globally. PREDICT updated and expanded the original “Hotspots” analyses of EID events (Jones et al. 2008), which revealed

increased risk of wildlife zoonotic and vector-borne EIDs originating in lower-latitude developing countries and correlations between emergence and human population density, and wildlife diversity (for zoonoses of wildlife origin). In this present research (“Hotspots II”) PREDICT researchers focused analyses on zoonotic diseases of wildlife origin, incorporated new data on EID events and additional hypothesized drivers of EIDs, and utilized new statistical modeling approaches to generate the following key findings and improvements over the original model.

Key Findings for Hotspots II

- Using new methodology, the Hotspots II model independently confirms some of the main conclusions of the original Hotspots I model, most notably that the risk for zoonotic disease emergence increases with higher mammal diversity.
- Mammal diversity, land-use type, and land-use change are the most important factors predicting emergence of zoonotic diseases of wildlife-origin. Hotspots I did not examine land-use type or land-use change, so this finding is novel (see Relative Influence Plot below). Specifically, the risk of EIDs increases with higher mammal diversity; certain land cover types, most notably several forest types; changes in the amount of land used for pasture; and higher levels of urban land cover (see Partial Dependence Plot below).
- Global maps of EID risk, adjusted for observation bias, confirm tropical regions as the most important EID hotspots (Figure 1). These regions often have greater forest cover, high biodiversity, large human populations, growing urban populations, and high levels of land-use change, all of which increase EID risk. Unadjusted data (not shown here) confirm that observations of novel EID events are still more frequent in higher latitude, developed countries where observation effort is the greatest.
- Africa, Asia, and South America have higher overall risk of EIDs after adjusting for observation bias, while Europe, North America and Oceania have lower overall EID risk (Figure 2). This finding nevertheless masks finer scale patterns of risk – all continents have some high and some lower risk regions (Figure 3).

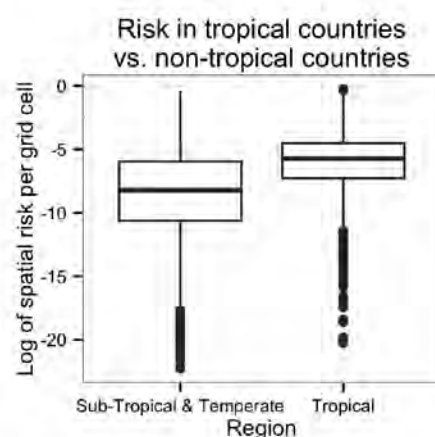


Figure 1. Risk of EIDs is higher in tropical regions relative to sub-tropical and temperate regions.

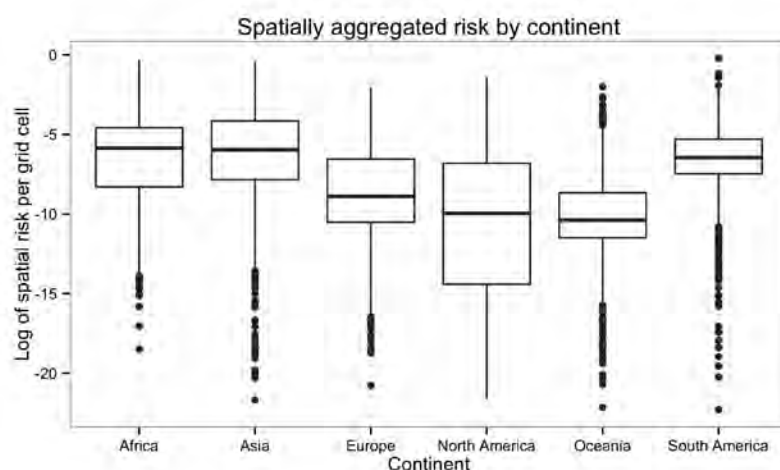


Figure 2. Spatially aggregated risk of zoonotic EID events by continent.

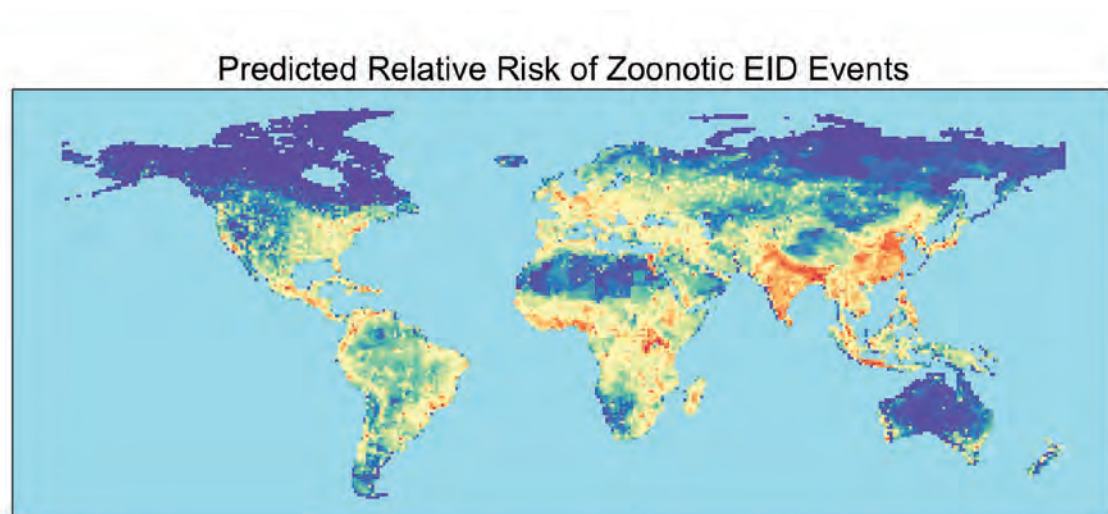


Figure 3. Hotspots II Map: Predicted relative risk of EID occurrence after adjusting for reporting bias. ‘Heat map’ scale Blue = lower risk through to Red = higher risk

Key Improvements in Hotspots II over Hotspots I

- The Hotspots II model is focused specifically on EID events caused by zoonotic pathogens of wildlife origin, which represent the majority of significant EID events and all recent pandemics. In addition, Hotspots II incorporated new data on hypothesized drivers of EID events, including habitat type, land-use, and land-use change.
- Change over time was incorporated into the Hotspots II model. While Hotspots I used 1990s population data for all EID events (a “snapshot in time”), Hotspots II slices population and certain land-use datasets by decade and uses the data from the most relevant decade for each EID event.
- Hotspots II explicitly accounts for the uncertainty in EID event location. In most cases, we do not know exactly where the first spillover or case cluster for an EID occurred, so the model was updated with an improved method of accounting for the spatial uncertainty in EID events.
- A machine learning approach (Boosted Regression Trees) was used for the Hotspots II analyses, which allows for relaxed assumptions and more effective analysis of non-linear relationships and interactions relative to traditional regression models. In addition, Hotspots II provides internal validation statistics to quantify model performance, greatly strengthening the interpretability of the findings.
- In the Hotspots II model, PREDICT quantified and accounted for reporting bias (i.e. observation bias; disease events are more frequently reported in developed countries) using an algorithm to search all journal articles in the PubMed Central Open Access Subset (n≈760,000 papers) for mention of specific locations (i.e. place names), whereas Hotspots I counted author addresses in a single journal (Journal of Infectious Disease, n=14,000 authors) at the country level. This revision resulted in a much broader swath of the biomedical literature being included, and provides spatial data at a much higher resolution (i.e. better than country level), allowing better estimates of the differences in reporting effort globally and a more refined estimate of EID risk.

Analysis of Drivers of Zoonotic Disease Emergence from Wildlife

In Hotspots II, PREDICT used two techniques to examine which hypothesized drivers of disease emergence are most strongly associated with zoonotic disease emergence from wildlife, and how each of these drivers is associated with increased EID risk:

Relative Variable Influence Plots. PREDICT generated plots of relative variable influence on disease emergence risk to assess the importance of various hypothesized drivers of EIDs (Figure 4). The plot shows the relative influence on EID risk (i.e. percentage of total EID event risk that a driver is associated with) for each hypothesized driver, with variables toward the top of the plot having the greatest importance. Variables were grouped into three broad categories, those representing human activities (e.g. land-use and population), animals (e.g. biodiversity and domestic animal reservoirs), and environment (e.g. habitat type and climate).

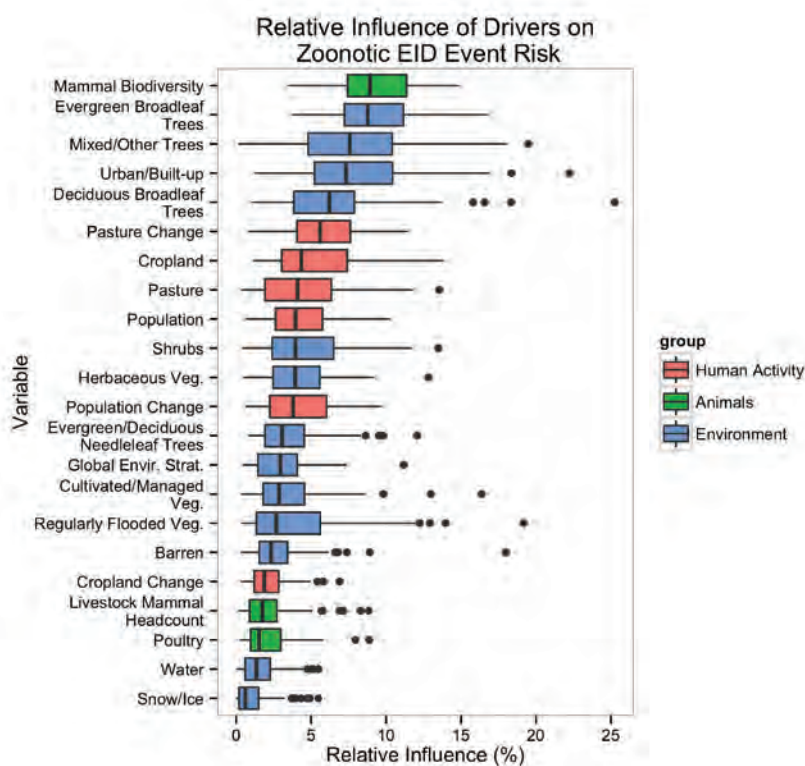


Figure 4. Relative influence of factors hypothesized to be important drivers of zoonotic EID events. The direction of influence is not explicit in this plot.

Partial Dependence Plots. PREDICT also generated plots to explain the degree and direction of influence each hypothesized driver has on disease emergence (Figure 5). Each driver (i.e. variable) is considered independently from the others (i.e. each plot shows the effect of a variable on the response after accounting for the average effects of all other variables in the model). These plots also show the uncertainty of each relationship – the thick line is a smoothed average across all model runs (i.e. those representing the spatial uncertainty of EID events) with color bands representing 95% confidence intervals. Variables were grouped into the aforementioned three broad categories (human activities, animals, and environment).

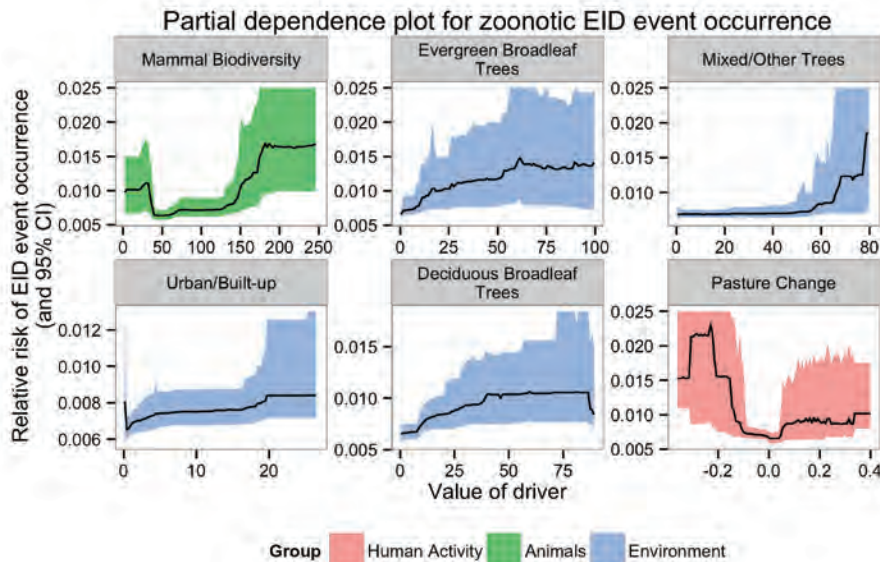


Figure 5. Partial dependence plots showing the six variables found to be most important for zoonotic EID event occurrence in PREDICT analyses. The x-axis represents the range of values for the driver (i.e. minimum to maximum) and the y-axis is the risk of an EID event occurring per grid cell (note different scales for each plot). The numbers are small because EID events are rare and their likelihood of falling in any one grid cell is very low. The lines show how EID risk changes with varying levels of each hypothesized driver.

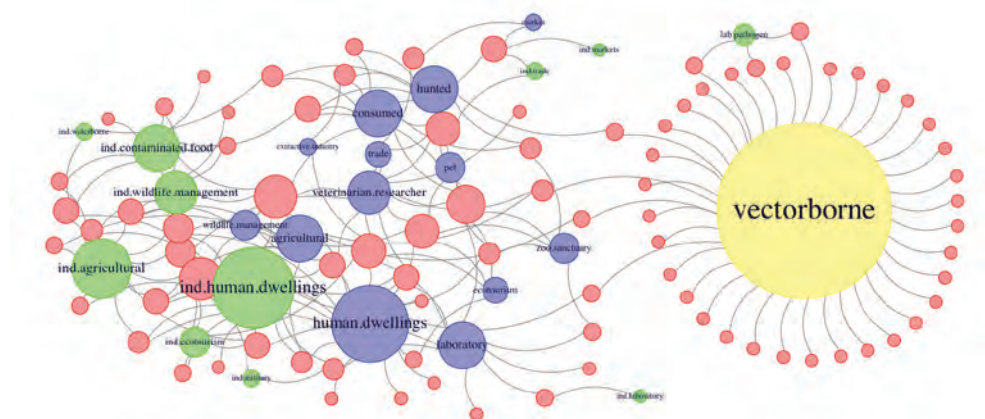
Results from the partial dependence plots revealed that:

- As mammal diversity increases in any given location, the risk of EIDs increases.
- Several habitat types (forests) show increased risk of EIDs as the percentage of each unit of area containing them increases.
- There is a marginal increase in the risk of EID events in more urban areas.
- Both pasture loss and gain influence risk of EID events, with minimal risk when pasture change is not occurring. From left to right, the x-axis represents negative change (i.e. losses of pasture area to another land use type), to zero change (i.e. no net change in pasture area), to positive change (i.e. increases in the areas used for pasture). Pasture change is measured in ten year time slices: 1970-80-90-2000.

HIGH-RISK INTERFACES FOR DISEASE EMERGENCE AND SPREAD

PREDICT conducted a systematic survey of all published data available through 2010 to identify the animal hosts, human activities, and high-risk disease transmission interfaces implicated at the point of human-animal contact and zoonotic disease spillover for all zoonotic viruses recognized to date. We used network analyses to examine common transmission pathways shared by animal hosts and high-risk interfaces, and model viral traits, host species and high-risk interfaces related to host taxonomic range, human-to-human transmission, and geographic distribution of viruses in order to provide insight into virus characteristics and conditions likely to pose risk for future disease emergence (Johnson et al. 2014).

- Disease transmission to humans involved wild animals for over 90% of zoonotic viruses, compared to one-third of viruses transmitted to humans from domestic animals. The vast majority of zoonotic viruses recognized to date are RNA viruses. Most zoonotic viruses were associated with spillover hosts from multiple wild animal taxonomic orders and domesticated species (i.e. livestock and companion animals). Network analyses revealed high host plasticity (i.e. broad host taxonomic range) among domestic and wild animal zoonotic viruses. Wild rodents were most frequently implicated in transmission of zoonotic viruses, particularly arenaviruses and bunyaviruses, while primate spillover hosts were commonly reported for zoonotic retroviruses, and bats for zoonotic paramyxoviruses and rhabdoviruses. Most zoonotic viruses with wild bird hosts were vectorborne.
- High-risk interfaces for zoonotic viruses transmitted by direct and indirect contact transmission included contact with wild animals in and around their dwellings and in agricultural fields (Figure 6). Occupational exposure associated with working with wild animals (veterinarians, researchers, and workers in laboratories) was also frequently reported. Primates were most commonly implicated in transmission to humans by direct contact during hunting and at laboratories, while rodents were more likely to be implicated in transmission by indirect contact in and around human dwellings and in agricultural fields.



Epidemiologic Circumstances Enabling Zoonotic Virus Transmission and Spread

- Zoonotic viruses that infect a broad taxonomic range of wildlife hosts were more likely to be capable of amplifying disease spillover by secondary human-to-human transmission and have broader geographic spread.

- Analyses revealed higher host plasticity in viruses transmitted at high-risk settings with mixing of phylogenetically diverse species in confined spaces, such as wild animals sold at markets or maintained in sanctuaries or zoos. Similarly, vector-borne viruses were reported in three times the number of host taxonomic groups than non-vector-borne viruses, likely because vectors also enable opportunities for cross-species disease transmission among hosts not normally in contact with one another.
- Results indicated that viruses with high host plasticity are especially prone to spillover, subsequent disease amplification by human-to-human transmission, and spread on a global scale. By identifying animal hosts, human practices, and high-risk interfaces acting synergistically to facilitate recent cross species disease transmission, we provide evidence for epidemiologic mechanisms that have long been suspected to promote disease emergence. Viral discovery efforts targeting settings with diverse host assemblages and control strategies focused on human practices facilitating cross-species disease transmission and direct contact with wildlife will have the greatest potential to limit future threats to public health.

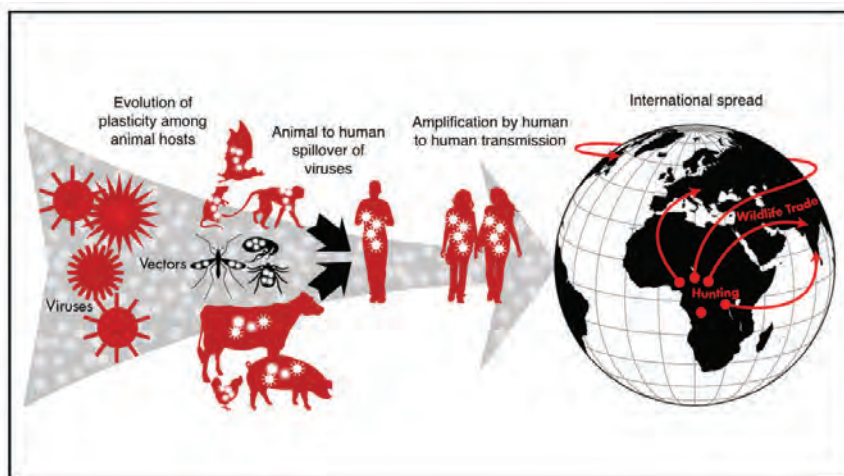


Figure 7: Illustration of key features in the progression of zoonotic virus emergence, spillover, amplification, and global disease spread. Human practices that promote transmission of mutation-prone RNA viruses able to infect a wide range of taxonomically diverse hosts, including wild and domestic animals, act synergistically to facilitate viral emergence, particularly for viruses capable of human-to-human transmission and broad geographic spread. From Johnson et al. 2014.

SICKI GRID PROJECT

Sicki GRID (Global Repository of Infectious Diseases) is a user-friendly and interactive website describing 372 novel EID events between 1940 and 2013. Sicki GRID phase I is targeted towards scientists, researchers, and health care professionals interested in infectious disease emergence, which in phase II will become an educational research tool for the general public as well. Data were collected across more than 40 variables, curated by PREDICT researchers, and described below. All information was extracted directly from the literature and is displayed in maps, tables, and short narratives. Features of the website include individualized event pages, a flexible search engine, map views, concise tabular data, narratives, and references (Figure 8).



Figure 8. Sample EID event page from the interactive SICKI GRID website.

Variables for EID Events in SICKI GRID:

- Date and location: start date, end date, duration, and location
- Pathogen: ICD10 classification, pathogen name, pathogen type, pathogen taxonomy, disease name, and drug resistance
- Impact: number of infected, dead, and hospitalized and prevalence of infected
- Clinical information: symptoms, disease, and sample type
- Transmission: general transmission, event transmission, zoonotic type, and domestication status
- Host information: initial host reported; host species name; host age; whether the host species is hunted, used as livestock, kept as pets, or another purpose; and host sex
- Economics: occupation, age of infected, age of dead, trade or travel restrictions, and per capita GDP and average lifespan in year and location of event
- Emergence: driver of emergence and EID category

CLIMATE CHANGE AND EID RISK: CASE STUDY OF HENIPAVIRUSES IN BATS

Prior studies on Nipah virus suggest that it emerged into the human population as a result of complex interactions between wildlife reservoirs (*Pteropus* spp.; fruit bats) and intensively managed livestock. Yet the emergence of this and other henipaviruses likely involves a suite of anthropogenic environmental changes, socioeconomic factors, ecological drivers, and changes in demography that overlay and interact with the distribution of these pathogens in their wildlife hosts.

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. We show that the distribution of henipavirus reservoirs, a proxy for the potential distribution of henipaviruses including Nipah and Hendra viruses, will likely change under a changing climate. We assessed the variation among climate models to find consensus about where climate change is most likely to lead to elevated risk from henipaviruses due to expanding host ranges, where risk will remain relatively stable, and where risk could decline as a result of shrinking host ranges

(Figure 9). Results from these analyses show that climate change is likely to be a key player in the future risk for henipaviruses due to the influence these changes could have on the distributions of reservoir bat species. This finding has important implications for managing public health risks and understanding drivers of disease emergence into the future.

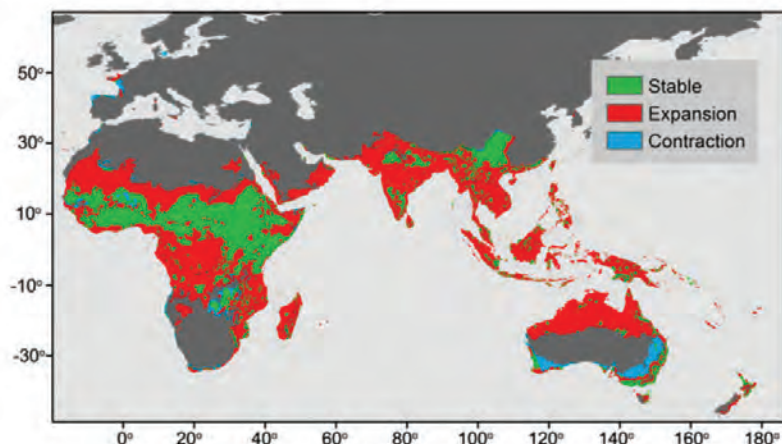


Figure 9. Synthetic generalization of the predicted expansion and contraction potential of climatic habitat for the midcentury A2 emission scenario based on 20 global climate change models and 13 bat species. Because each species was modeled individually, expansion is defined as the presence of at least one species and no change in the other species, and contraction was defined as the absence of at least one species and no change in the other species

HOST-PATHOGEN PHYLOGENY PROJECT: IDENTIFYING HOST AND VIRUS TRAITS TO PREDICT ZONOOSES

To investigate how host and virus traits influence the risk of emergence, PREDICT created a database of all known mammal viruses published in the International Committee on Taxonomy of Viruses (ICTV) v8 assessment and every known mammal host from the published literature associated with those viruses. This database included 2,738 mammal-virus associations, 768 unique mammal species, and 593 unique viruses from 29 viral families.

We then collected data for a wide range of traits for each host and virus in the database. From a mammal host perspective, data collection included genetic relatedness to humans; area and percent of host range covered by crops, pasture, or urban areas; whether hunted or not; domestic species status; population density across host range; and number of total known viruses and scientific publications for each species (bias measures). From a virus perspective, we collected taxonomic and basic viral traits for each virus including RNA/DNA, segmented or non-segmented, replication in the cytoplasm, genome length, and a measure of host breadth. PREDICT explored sets of all possible models based on the variables above and used Generalized Additive Models (GAMs) to identify what factors help predict the number of viruses that each host shares with humans.

The analyses confirmed that PREDICT target taxonomic orders of nonhuman primates, rodents, and bats have the greatest risk of sharing viruses with humans. Non-domesticated ungulates (Cetartiodactyla and Perissodactyla) and carnivores have a relatively lower risk. Also, as phylogenetic distance from humans increases within each order, the risk of sharing viruses goes

down (Figure 10). The best model predicting the number of viruses a given mammal species shares with humans explained 87.5% of the deviance, has an adjusted R-squared of 79.4%, and includes the following variables: total number (logarithm) of viruses found in the focal mammal species, phylogenetic distance from humans of the focal mammal species, mass in grams (logarithm) of focal mammal species, median human population density (logarithm), percent of the focal mammal species range that is urbanized, and number of citations (logarithm) in Zoological Abstracts regarding the focal mammal species.

In Summary:

- Wildlife species most closely related to humans, with the highest pathogen diversity, and greatest geographic overlap with humans (e.g. urban areas) are the most likely to host a virus that emerges in people (i.e. are associated with the highest risk for future virus emergence);
- Bats, rodents, and primates historically share the greatest proportion of viruses with people; other mammal hosts are also important (Figure 11); and
- Research effort has historically biased our knowledge of virus diversity, highlighting a need for more standardized disease surveys in wildlife.

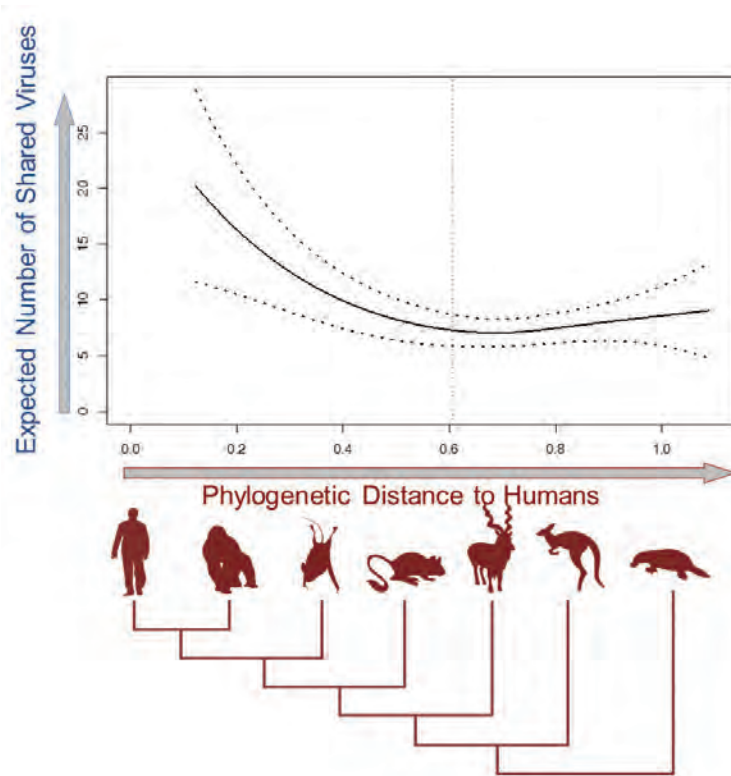


Figure 10. The number of viruses shared with humans depends critically on the species' phylogenetic distance from humans for non-domesticated mammals. This model controls for research effort based on the number of disease-related citations for each species.

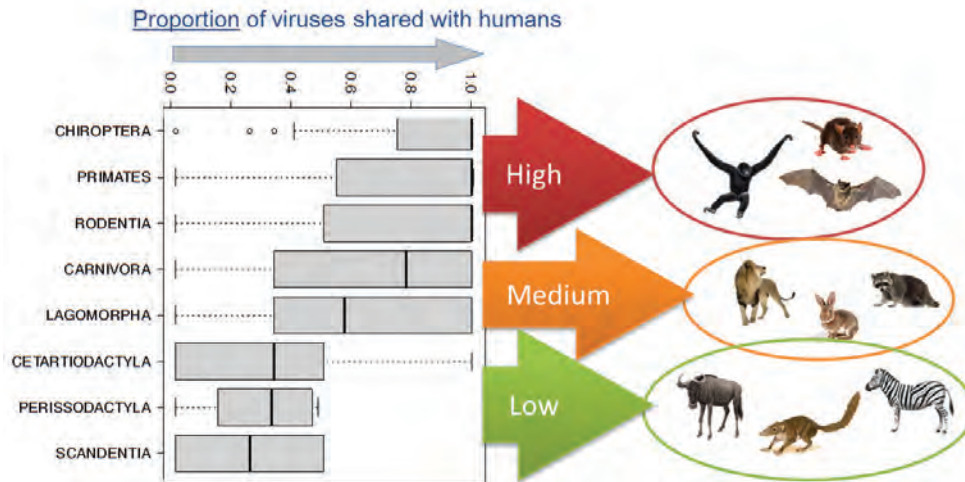


Figure 11. Some taxonomic groups of mammals, particularly bats, rodents, and nonhuman primates, have a very high proportion of viruses shared with humans when data was analyzed at a species level. This is notable given that the number of total known viruses is not significantly different across groups.

VIRODIVERSITY: A STRATEGY TO ESTIMATE UNKNOWN VIRAL DIVERSITY IN MAMMALS

Despite the importance of wildlife as reservoirs for many EIDs in humans, there are no estimates of the viral diversity that exists in even a single mammalian wildlife species. PREDICT sought to provide such an estimate for one wildlife host, the Indian Flying Fox (*Pteropus giganteus*), which is known to be the source of at least one important emerging infectious disease (Nipah virus).

Using PREDICT diagnostic laboratory protocols, bats were repeatedly sampled to discover and analyze the patterns of 55 viruses from nine viral families (Anthony et al. 2013). Biodiversity analytical techniques were then used to estimate the total viral richness for all viruses (Figure 12 A) and for each viral family (Figure 12 B), and to estimate the number of as yet undetected viruses existing in this host species. We also used a simple extrapolation to estimate the order of magnitude of the mammalian virosphere (~300,000 viruses across nine viral families across all species of mammals) assuming other species had a similar number of viruses and that there was no viral overlap between species (Anthony et al. 2013). While this first estimate needs refinement with more data from more species and from more regions, PREDICT estimated that the cost of detecting the majority of these viruses (\$1.6 billion) would be much less than the cost of a single pandemic emergence event (e.g. SARS \$30-100 billion). This has implications for how we might plan to reduce EID risk in the coming decade, as early discovery of viruses that may later become pandemics could facilitate prevention or early mitigation.

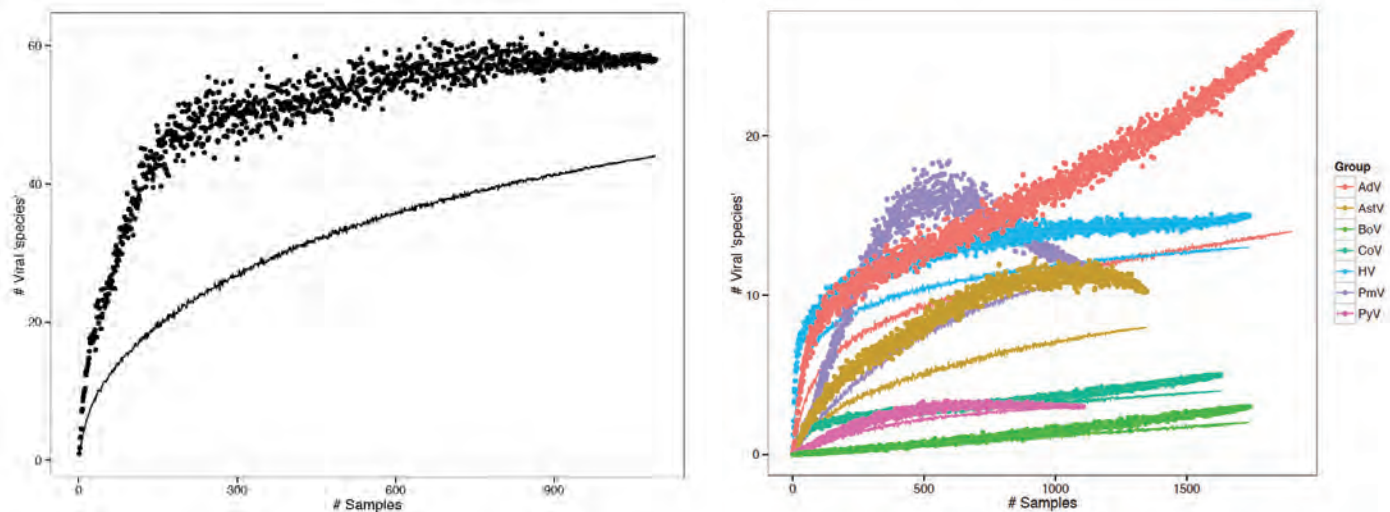
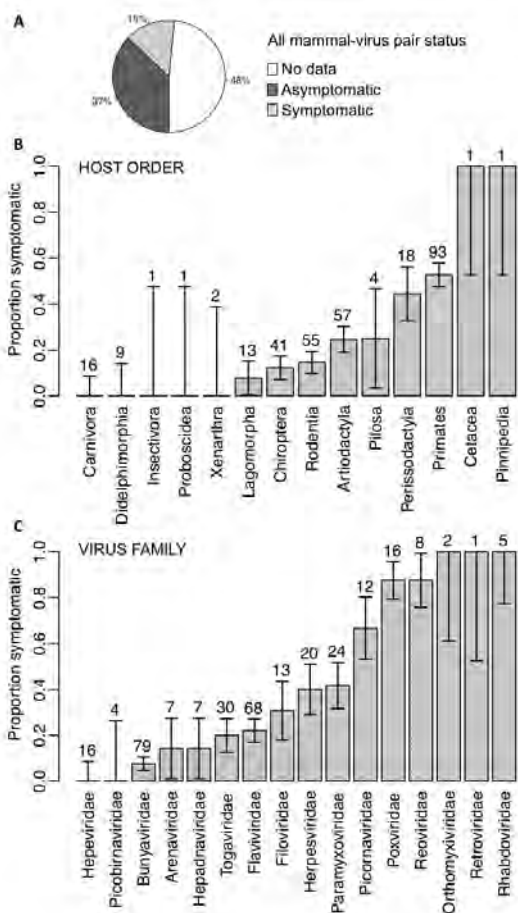


Figure 12. The observed number of viruses (solid lines) and the estimated number of viruses (dots) with increasing sampling effort (number of samples screened). A) all viruses, B) viral family level. The curves (solid lines) show that detecting viruses follows a trajectory of diminishing returns. Estimates (dots) show that the total number of viruses can be estimated with considerable stability in some cases well before maximum sampling size is reached (and in some cases more sampling effort is required). Such analytics pave the way for more efficient viral discovery in future studies.



TARGETING VIRAL DISCOVERY IN SYMPTOMATIC VS. ASYMPTOMATIC ANIMALS

PREDICT created and analyzed a detailed database of 605 mammal-virus associations for emerging disease events previously published in Jones et al. (2008) to evaluate whether surveillance targeting diseased animals is the best strategy to identify potentially zoonotic pathogens (Levinson et al. 2013). The findings suggest a mixed healthy and diseased animal surveillance strategy is generally best, although surveillance of apparently healthy animals would likely maximize zoonotic virus discovery potential for bats and rodents. In addition, the animal host condition and health were only reported in half of all investigations (Figure 13 A), highlighting the need for better data collection during wildlife disease investigations. Further, the percentage of symptomatic hosts with viruses varied by host order (different groups of mammals more likely to be symptomatic) and by viral family (Figure 13 B and 13 C).

Figure 13. A) Percentage of host-virus pair reports describing symptomatic (observable) disease, asymptomatic disease (no observable disease), or no data (no description of disease included). B) Proportion of symptomatic hosts by mammal taxonomic order. C) Proportion of viruses, by taxonomic viral family, for which hosts were reported symptomatic. The total number of each host order or virus family included in the database is given above each bar. SEs (error bars) were calculated assuming binomial error structure. Adapted from Levinson et al. 2013.

VIRAL SURVIVAL: PATTERNS IN ENVIRONMENTAL PERSISTENCE

The risk of viral emergence and spread is directly linked to the frequency and extent to which a person comes into contact with a pathogen. A subset of this risk that is not fully documented is the risk posed by free virus in the environment – shed virus either from humans or animals. PREDICT analyzed published literature and experimental studies on viral survival to identify patterns and important factors influencing viral survivability outside of the host. This analysis confirmed that viral survival can be greatly affected by temperature, relative humidity, and surface type. Viral detectability in these experimental studies varied immensely, as different study methodologies were employed; furthermore, detectability did not relate to viral infectivity, which when tested is significantly lower than documented half-life (time to halve the number of viable viruses) values. In addition, virus half-life varied among seven key viral families, but was typically less than 48 hours. Influenza viruses and herpesviruses were notable exceptions, especially when tested on non-natural surfaces.

WHAT-IF SCENARIOS

For emerging infectious diseases (EIDs), it is critical to understand their potential for spread and how to best prevent it. In order to form a scientific basis for understanding how best to stop the spread of the potential EIDs identified under the PREDICT project, we developed detailed models of the spread of eight pathogens, including GB virus (a hepacivirus formerly called hepatitis G) in Bangladesh, Bas Congo virus in the Democratic Republic of Congo, hantavirus in China, herpes B virus in Malaysia, MERS-like coronavirus in Egypt, simian foamy virus in Cameroon, SARS-like coronavirus in Thailand, and a paramyxovirus in Indonesia. These case studies represent a diversity of transmission modes from zoonotic source to humans (direct, blood-borne, vector-borne), as well as modes of human-to-human transmission (direct, blood-borne, vector-borne). Spillover was modeled as coming from the key zoonotic taxa of bats, rodents, and nonhuman primates and from contact involving hunting, consumption from markets, guano use, and other non-consumptive interactions. PREDICT also examined the potential for human-to-human transmission to spread the disease globally by air travel (Figure 14).

We used specific known spillover locations or identified a most likely candidate location, where a number of positive samples had been collected. We then used information on population density, both in identified spillover locations and the nearest urban center with available air travel to parameterize the scenario. Both spillover and human-to-human transmission were modeled based on known transmissions or the mode of transmission for a closely related pathogen. Our cases and interventions were pulled from a survey of literature combined with expert knowledge relevant to the particular case studies.

Three critical conclusions were reached:

- Reducing exposure to the zoonotic source was almost always the most effective intervention.
- Culling wildlife was never a very successful strategy to reduce spillover risk.
- Due to global travel and trade, the United States was almost always one of the top twelve countries at risk of imported cases.

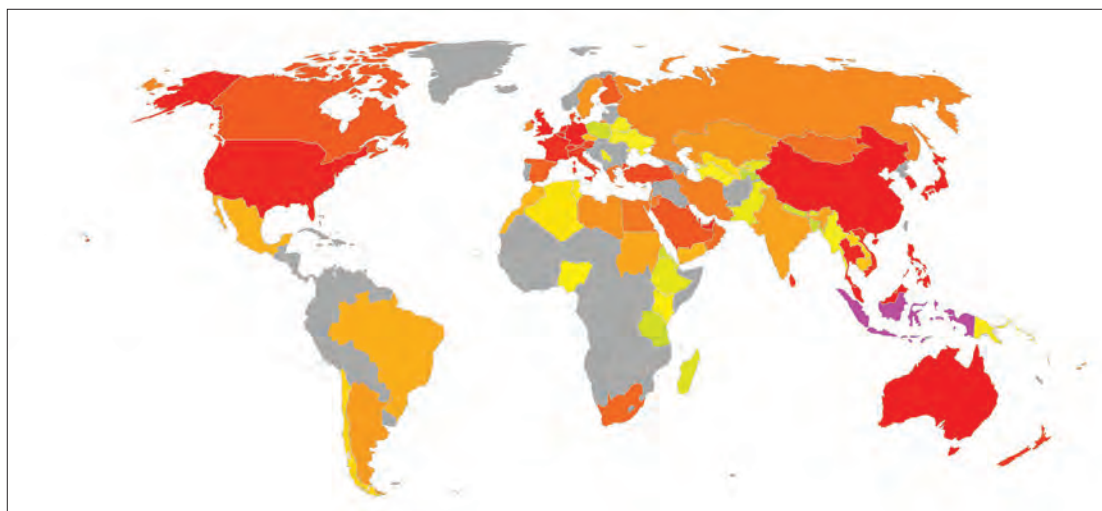


Figure 14: What-if scenario of the spread of a paramyxovirus from Indonesia showing the 127 countries that can be reached by flights of two legs or less based on airline travel data (number of passengers) from 2013 and the methods in Hosseini et al. 2010. RED indicates earliest arrival; YELLOW, intermediate; GREEN, latest arrival; GREY, countries that cannot be reached from origin without more than two legs of air travel; PURPLE = hypothesized origin of flights and ground zero for outbreak.

TRAVEL AND TRADE: GLOBAL VULNERABILITY TO EID EMERGENCE AND SPREAD

Previous EID “hotspots” maps (Jones et al. 2008) show the relative risk of a novel disease originating in a region. If we are concerned about pandemic risk, we also need to know how likely it is for the disease to be propagated from a region to become a pandemic. Often, pandemic spread will be a product of the volume of people traveling into and out of a region, considering the region’s underlying hotspot potential.

We demonstrated that air travel data, in combination with healthcare resources, can successfully predict the reported global spread of Influenza A H1N1 – a pandemic respiratory pathogen (Hosseini et al. 2010). PREDICT used this approach and constructed an index (equation 1, below) to incorporate the number of airline passengers that connect the source (i) and target (j) locations (C_{ij}), the Jones et al. hotspots predictions to provide a relative risk of an EID emerging in a source location (E_i) and healthcare spending per capita to index the likelihood of an EID being halted at the source location (H_i). Thus, ϕ_i is the index of vulnerability to the spread of all potential EIDs from all hotspots. This risk is generated for every airport, and we then interpolate this risk based on a distance weighting method for every year of available air travel data from 2006 to 2013.

PREDICT used this approach to produce maps that demonstrate how the risk of EID emergence and spread changes over the 2006 to 2013 time period (Figure 15). These analyses illustrate that South, East, and Southeast Asia remain major areas of EID vulnerability, as air travel and trade is one means that EIDs could spread through and among these regions. In addition, Africa is becoming increasingly connected to the rest of the world, expanding both its vulnerability to and risk as a source of EIDs.

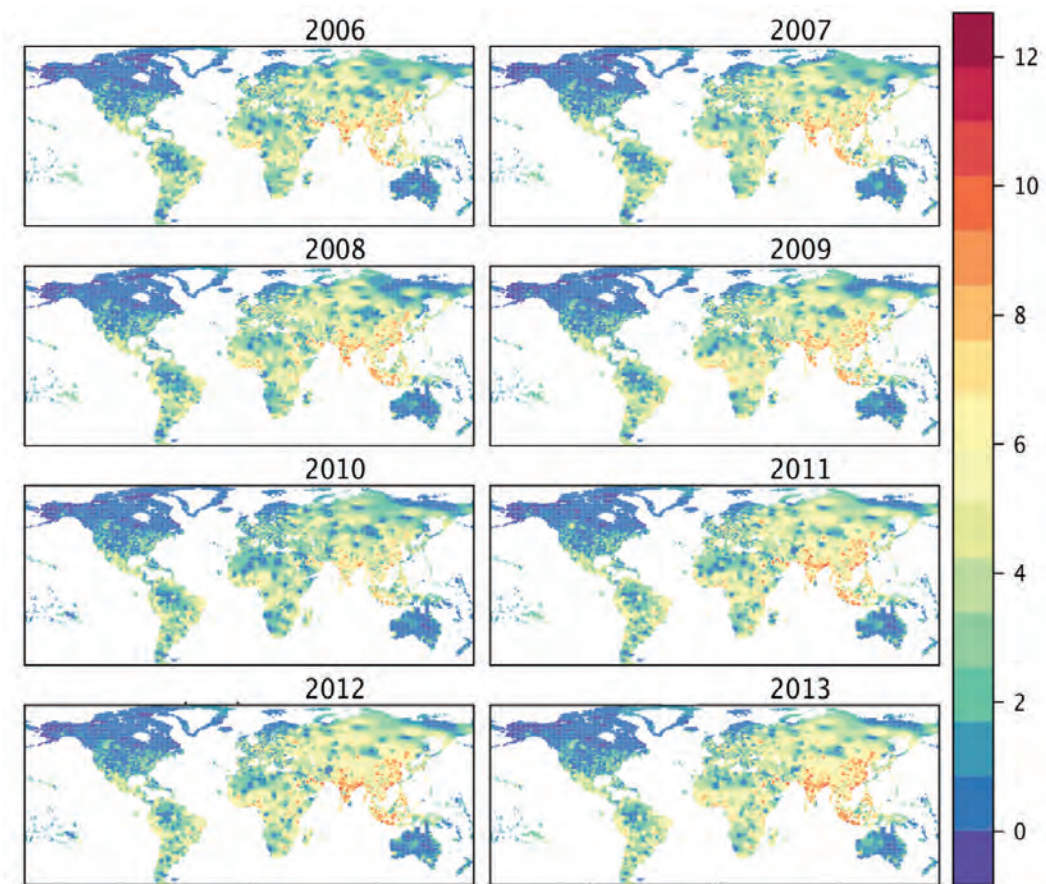


Figure 15: Global vulnerability to the spread of EIDs based on Jones et al. 2008 Hotspots and air passenger data using Equation 1 below.

Equation (1)

$$\phi_j = \sum_{all i} \frac{C_{ij} \cdot E_i}{H_i}$$

Travel and Trade – Case Studies: H7N9, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Ebola virus

PREDICT also used airline travel data to predict the order of arrival of Influenza A/H7N9, MERS, and Ebola-infected passengers globally based on Hosseini et al. 2010 (Figure 16). These maps identified the hypothesized most at-risk countries for MERS and Ebola, with good concurrence to the subsequent spread of both diseases.

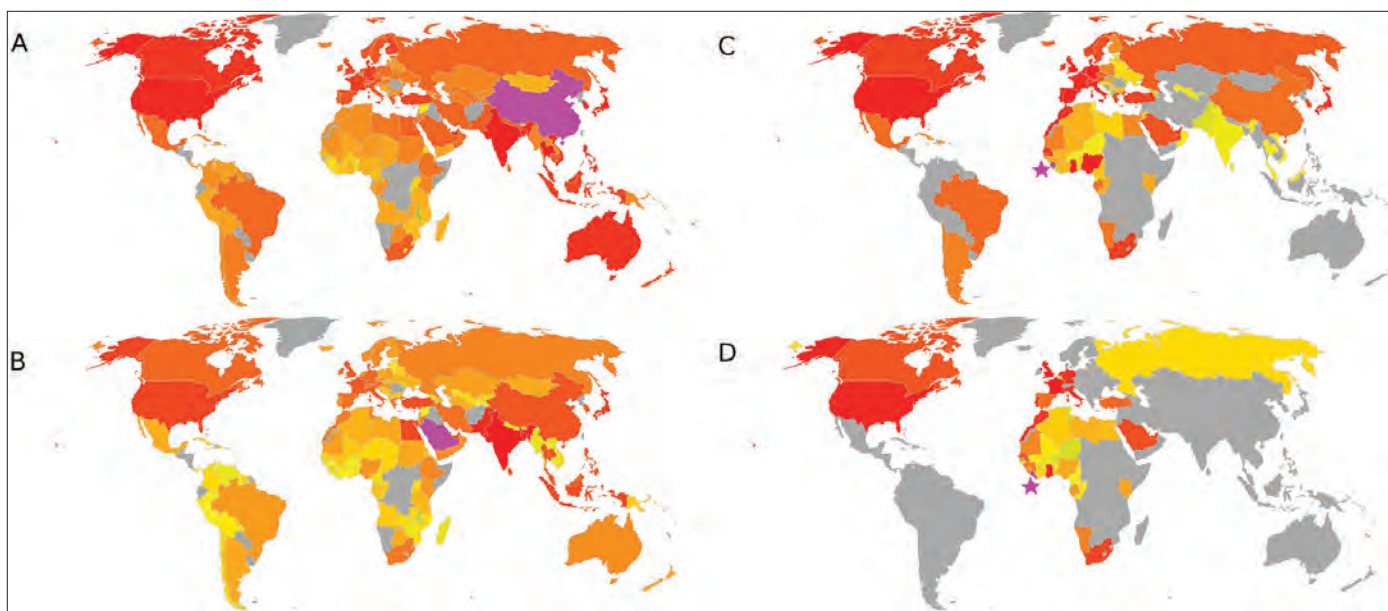


Figure 16: Potential order of spread of (A) Influenza A/H7N9 from China, (B) MERS from Saudi Arabia, (C) Ebola virus from Sierra Leone, and (D) Ebola virus from Liberia. PURPLE = origin of flights and ground zero for outbreak without more than two legs of air travel; PURPLE star adjacent to country of origin in (C) and (D); RED = earliest arrival; YELLOW = intermediate; GREEN = latest arrival; GREY = countries that cannot be reached from origin without more than two legs of air travel.

TRANSMISSION PATHWAYS: TARGETING ZONOSSES SURVEILLANCE AND CONTROL

An important challenge in developing effective surveillance and control strategies of zoonotic EIDs is identifying the relevant transmission pathways (or routes) that allow for pathogen transmission between host reservoirs and humans. Zoonoses can be transmitted via a range of routes (or pathways), yet research exploring the role of transmission pathways in past EID events had been largely unexplored. PREDICT developed a novel approach targeting key transmission pathways at the human-animal interface in order to identify the potential ways by which a pathogen might spillover from wildlife host reservoirs into human hosts. We used this approach to analyze the most important transmission pathways (e.g. vector-borne, direct animal contact, airborne) identified in previously reported emerging infectious disease events. Our analysis suggests that at the broad scale, the likelihood of transmission occurring through any one pathway is approximately equal. However, the major transmission pathways for zoonoses vary widely according to the specific underlying drivers of EID events (e.g. land-use change, agricultural intensification; Figure 17). Our method provides insights into which transmission pathway may be more likely to occur with different underlying drivers, thus allowing for more targeted prevention measures and surveillance approaches.

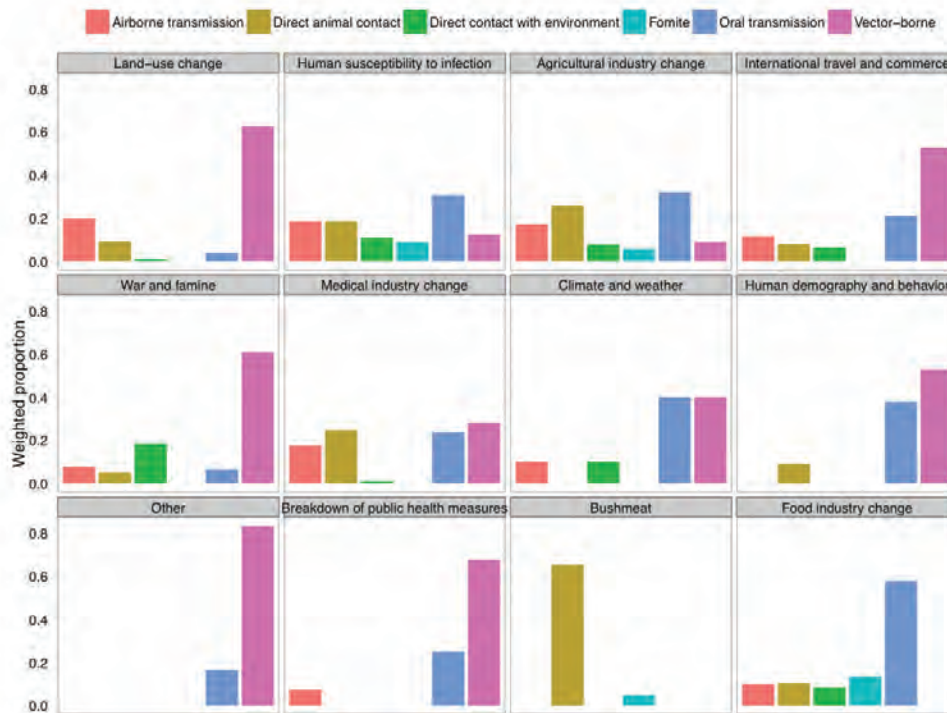


Figure 17. Proportion of zoonotic EID events (n=180), from a known database of 335 EID events from 1940 to 2004 (Jones et al. 2008), per transmission route categorized by the primary driver of disease emergence for each pathogen. For example, disease emergence events in regions under pressure from land-use change have most often occurred through the vector-borne pathway, airborne transmission, and, less so, through other routes of transmission. Alternatively, for bushmeat consumption, the only relevant transmission pathways are direct animal contact and contact with contaminated fomites.

STRATEGY TO RANK ZOOONOTIC POTENTIAL OF NOVEL VIRUSES

The majority of the viruses detected by PREDICT were not isolated and have been identified only through analysis of partial genetic sequences (300-1000bp gene fragments). Using their genetic sequences, we can determine relationships with known viruses in animals and people. However, without full genome sequencing, or virus isolation followed by a series of experiments, it is difficult to determine a virus' zoonotic potential. Thus, a challenge remains in moving towards a predictive framework to estimate spillover risk for viruses. PREDICT is integrating multiple approaches to prioritize new viruses detected for further characterization and examination of spillover risk. We use data published in the literature on known zoonotic viruses and virus-mammal associations to develop a series of independent emerging infectious disease (EID) risk models and other metrics to quantify spillover risk from newly discovered but not fully characterized viruses. We combined all available data published on recognized viruses, along with host data collected by field teams while conducting surveillance activities, to parameterize metrics used in ranking. The data will include both virus-dependent factors (i.e. based on sequence or viral family-level traits) and virus-independent factors (e.g. geographic location, host species identification and abundance, host plasticity, viral prevalence, and animal-human contact interface) to assess spillover potential (Figure 18). We also evaluate the sequences detected in the context of phylogenetic relatedness to known pathogens, detection of a virus sequence in an unusual host or previously unrecognized high-risk interface, and associations with undiagnosed illnesses in people or animals sampled by PREDICT.

Summary of Data to Estimate Zoonotic Potential for Novel Viruses

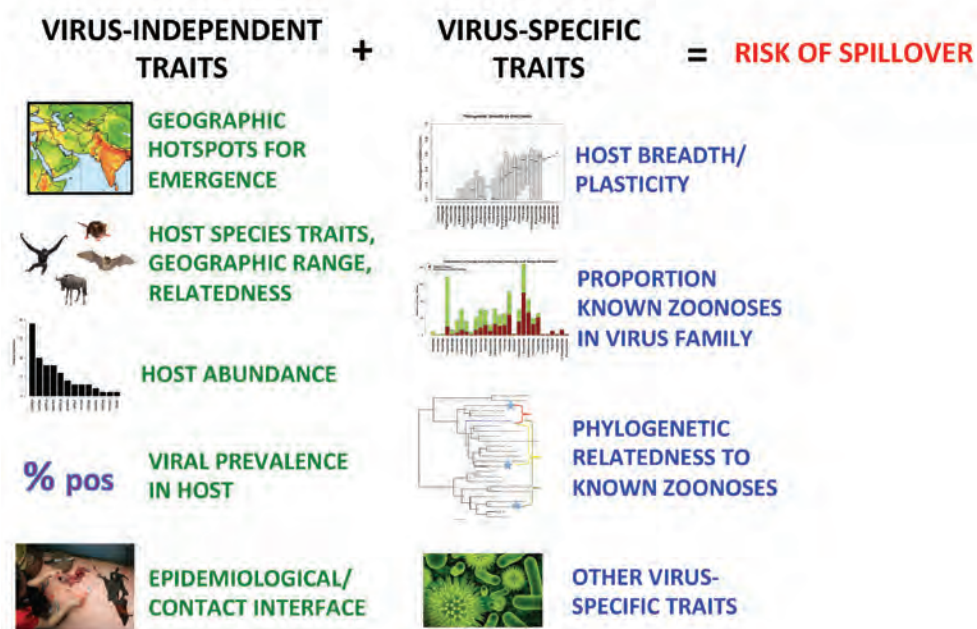


Figure 18. Virus-independent and virus-dependent data used to derive viral spillover risk.

ECONOMIC ANALYSIS OF PANDEMIC MITIGATION VS. ADAPTATION

Emerging pandemics can have a very significant impact on global economies, especially through disruption of travel and trade networks (e.g. SARS which cost \$10-50 Billion). These costs are likely to rise exponentially in the future, because the number of EIDs emerging each year is increasing by about five new emergence events. Furthermore, in an increasingly globalized world, the probability of a disease that emerges in a hotspot country rapidly affecting those with high dependence on trade and those contributing significantly to the global economy (i.e. high gross domestic product (GDP)) is also increasing over time.

For analysis, we framed an analogy between pandemic emergence and global climate change. Globally-coordinated strategies to address climate change are classed as either: 1) adaptation, in that they continue business-as-usual and assume we will design strategies to reduce their impact; or 2) mitigation, in that they attempt to reduce CO² levels and reduce climate change. For pandemic emergence, our current global control strategies are largely adaptive, in that they attempt to reduce the impact of a pathogen after it has emerged by conducting outbreak investigation and control programs, supplying drugs and vaccines, and other measures. Programs like USAID-EPT can be considered mitigation because they aim to reduce pandemic risk before significant human-to-human spread.

PREDICT used the same modeling strategy that informs the debate over adaptation and mitigation for climate change called “real options economic modeling,” which allows for evaluation of multiple mitigation strategies across various scenarios with regard to program costs and benefits. We used data on the cost of current globally-coordinated adaptation strategies for pandemic prevention. We showed that such strategies would be optimally implemented within 27 years to reduce the annual rise of EID events by 50% at an estimated one-time cost of approximately \$343.7 billion. With

significant delays in achieving agreed-upon capacity for implementation of the International Health Regulations (a globally-coordinated control program), and adoption of the Global Health Security Agenda to deal with this lack of capacity, the conclusion is that these adaptation strategies need to be executed extremely rapidly to function optimally.

We then analyzed World Bank data on multilateral “One World-One Health” pandemic mitigation programs. The analyses revealed that because most pandemics have animal origins, mitigation is a more cost-effective policy than business-as-usual adaptation programs, saving between \$344.07 billion and \$360.3 billion over the next 100 years if implemented today. We conclude that globally-coordinated pandemic prevention policies need to be enacted urgently to be optimally effective and that strategies to mitigate pandemics by reducing the impact of their underlying drivers are likely to be more effective than business-as-usual.

QUANTIFYING ECONOMIC IMPACTS OF EIDS: CASE STUDY OF SARS

The economic damages of EIDs can be very high (e.g. SARS – estimated \$10-50 billion), but these damages have only been estimated rarely and without standard methodology that:

- Is applicable to outbreaks that occur across multiple countries;
- Deals with the interconnectedness of economic sectors, wherein losses in one country or sector may be gains in another;
- Defines a relevant stakeholder – individual, economic sector, country;
- Specifies a relevant temporal window, as economic damages seen in the short-term may be compensated for in the long-term, as markets rebound; and
- Is rapid, which means it utilizes data that are readily available before, during, or shortly after an outbreak.

Using SARS as a test case, PREDICT developed a microeconomic approach to EID economic damages estimation in which the target stakeholder is “the household.” Household-level cost is defined as the cumulative outlays paid by individuals (or countries/insurance companies for those individuals) stricken with disease. This unit of analysis provides both specificity on exactly who bears the cost and the exact nature of that cost, as well as the flexibility to accommodate damages of disease across all countries in which infections were reported. Costs broadly follow the number of cases but vary among countries with different levels of development (Figure 19 A). Identifying the bearers of cost (e.g. out of pocket costs incurred by individuals, Figure 19 B) facilitates the leverage of incentives at the population level for EID risk reduction investments. Our method yields a consistent, reproducible, and relative estimate of economic damages attributable directly to the presence of an EID and lays a foundation for relative impact assessments between diseases, among stakeholders, and through time.

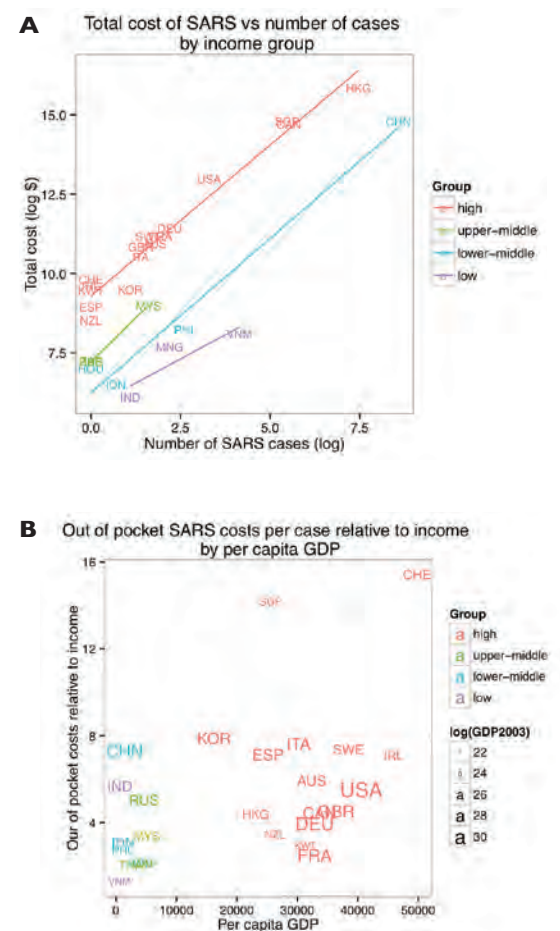


Figure 19. A) Cost of SARS cases vs. number of cases, split out by country income level (low, middle, high); B) Out of pocket costs per case relative to income by per capita GDP.

DEMOGRAPHIC CONDITIONS FOR OUTBREAKS: CASE STUDY OF EBOLA IN WEST AFRICA

Preliminary Analysis of Initial Conditions of Ebola Outbreaks

While not occurring in PREDICT-supported countries, the 2014 West African Ebola virus disease (EVD) epidemic is crucial to our understanding of mitigation of EIDs in areas where the governments are not expecting them to occur or prepared for their response. This outbreak had seen over 9,000 cases – more than 15 times the size of the next largest EVD outbreak – and showed no sign of abating at the time of this publication (September 2014). However, it is not known whether this outbreak appeared to be different from previous outbreaks at the outset in a way that could explain its unprecedented magnitude. PREDICT formed two explanatory hypotheses:

1. This outbreak might have initially occurred in a more densely populated area than others. This scenario could have affected transmission dynamics (e.g. increasing rate of transmission) and could have led to the more severe epidemic.
2. On the other hand, this epidemic might have been discovered later on in its natural progression, provoking a later response and allowing the disease to better establish itself in the human population prior to the implementation of response efforts.

We gathered data on eight EVD outbreaks with over 100 cases, including the 2014 epidemic. For each outbreak, we recorded from the literature: initial location of emergence, date of initial report, initial number of cases and deaths reported, and total number of cases and deaths (Table 1 and Figure 20).

Table 1: Historical Ebola virus disease outbreaks with >100 cases.

Region	Likely Country of Origin	Region Pop. Density	Date of Recognition	Initial Cases	Total Cases	Total Deaths
Nzerekore, Faranah	Guinea	39.9	3/22/2014	80	9911 ¹	4868 ¹
Western	Uganda	148.9	11/30/2007	50	149	37
Kasai-Occidental, Bas-Congo	Democratic Republic of the Congo	29.6	9/11/2007	372 ²	264	187
Cuvette-Ouest	Republic of the Congo	2.7	2/5/2003	61	143	128
Northern	Uganda	60.3	10/8/2000	51	425	224
Bandundu	Democratic Republic of the Congo	16.6	5/10/1995	100	315	250
Western Equatoria	South Sudan	4.5	10/29/1976	NA	284	151
Equateur	Democratic Republic of the Congo	11.9	10/19/1976	17	318	280

¹As of October 22, 2014.

²Initial reported case numbers were revised downward over the course of the epidemic.

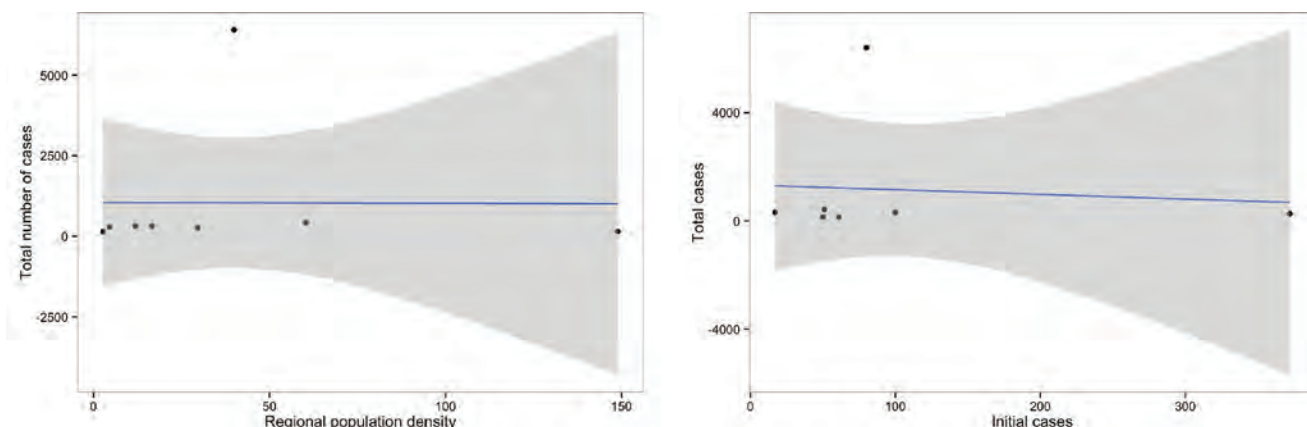


Figure 20. Demographic conditions of past Ebola virus disease outbreaks. Total/current cases represented on the y-axis. The left plot shows regional population density on the x-axis, and the right plot shows number of cases when discovered on the x-axis. Both plots display lines of best fit and confidence intervals for those lines. Basic linear models do not show an association between either regional population density or number of initial cases at discovery and total case count.


This preliminary analysis suggests that the 2014 EVD outbreak was not discovered under significantly different demographic conditions than the other significant EVD outbreaks. This result suggests that even smaller outbreaks should warrant a swift, large-scale response. We recommend further study to analyze the costs and benefits of larger-scale responses to all EVD epidemics and to explore other factors which could explain the magnitude of this epidemic in comparison to others.

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HIGHLIGHTS OF PREDICT PUBLICATIONS





As a testament to the success and utility of the PREDICT project and the work of its team of dedicated One Health professionals, findings from PREDICT work as of 2014 resulted in or contributed to more than 90 peer-reviewed, scientific publications that improve our understanding of zoonoses and the factors influencing their emergence.

Though manuscript publication in the scientific literature has never been a primary goal of the PREDICT Consortium, the wide distribution of the project findings that scientific publication facilitates is assisting in cutting-edge global health improvements, including surveillance science, diagnostic technologies, understanding of viral evolution, and ecological driver identification. In addition, peer-review publication serves to validate PREDICT's professional approaches on an international scale. We highlighted here PREDICT's contribution to the scientific literature within the following categories: surveillance strategy improvements; risk characterization; pathogen discovery and characterization; laboratory methodology and technology; influenza virus-specific characterization and surveillance strategies; contributions to global health policy; and other contributions to enhancing our understanding of zoonotic diseases.

The citations for publications stemming directly from the PREDICT project are indicated by bold and italic font and citations for publications that benefited from intellectual developments from the PREDICT project are indicated by italic font only.

SURVEILLANCE STRATEGY IMPROVEMENTS

Framework for PREDICT's Surveillance Strategy

PREDICT strengthened ongoing surveillance activities in global hotspots for emerging diseases in order to detect high-consequence pathogens circulating in wildlife populations and enhance our understanding of factors leading to increased risk of spillover, amplification, and spread. The framework for PREDICT's surveillance strategy was presented in a number of publications by PREDICT researchers (*Bogich et al. 2012a; Karesh et al. 2012a; Morse et al. 2012; Olival et al. 2013a*). *Morse et al. (2012)* outlined a series of research and surveillance opportunities and goals that could aid in the movement of the global pandemic strategy from response to preventive action. The authors conducted a systematic review of the literature to gather information on emerging pathogens, the factors associated with their emergence, and their hosts. They presented strategies to target surveillance at the most high-risk human-animal pathogen interfaces and discussed how technological advances in diagnostics, informatics, and mathematical modeling led to major improvements in capabilities for disease surveillance. The authors also discussed the challenges associated with cooperation and coordination of resources among the health, agriculture, and environmental sectors for prevention and control of zoonotic diseases. They

stressed the need for an integrated multi-sectoral approach to understand the complex ecological and social changes that contribute to emergence of infectious diseases and to identify the best interventions to prevent spillover of zoonotic diseases into people (*Morse et al. 2012*).

In addition, *Karesh et al. (2012a)* provided the first comprehensive look at the causes and relationships of both endemic and novel emerging diseases, which account for over one billion human cases of illness annually. The authors reviewed the mechanisms by which zoonoses result from natural pathogen ecology and how other circumstances, such as animal production, natural resource extraction, and antimicrobial application change the dynamics of disease exposure in people. The authors discussed the utility of a coordinated and targeted approach to disease prevention and control, as well as the health and economic benefits of “upstream” early detection and response (*Karesh et al. 2012a*).

Bogich et al. (2012a) presented a framework for an integrated approach using targeted surveillance and modeling to make predictions about the future risk of emerging infectious diseases (EIDs) originating from wildlife. The authors proposed a strategy for a unified predictive model that addresses three stages of disease emergence: 1) “pre-emergence” where interspecies transmission of pathogens occurs among animal populations; 2) “spillover” where interspecies transmission of pathogens occurs between animals and humans; and 3) “pandemic emergence” where the emerging pathogens spread across continents (*Bogich et al. 2012a*). Lastly, *Olival et al. (2013a)* assessed the relationship between environmental stewardship and conservation and health and argued for a conservation-minded approach to prevention of zoonotic disease emergence; one that acknowledges the linkages between environmental destruction and the emergence of zoonotic pathogens.

Capacity Building for Surveillance of Zoonotic Viruses in Wildlife

We evaluated the capacity to conduct wildlife surveillance for potentially zoonotic viruses in PREDICT countries through ‘rapid survey’ questionnaires. The goals of the surveys were 1) to better understand local attitudes and perspectives regarding opportunities, challenges, and priorities for capacity building efforts and 2) to track in-country capabilities for virus surveillance in wildlife over time (*Schwind et al. 2014a*). Questionnaires were administered to wildlife officials and PREDICT in-country project scientists in 16 countries with the aim of comparing perspectives between the officials and scientists regarding needs for capacity development for surveillance of zoonotic viruses in wildlife. Both groups prioritized wildlife hunting and markets as critical targets for conducting surveillance and identified a lack of sustainable funding as the most significant challenge for conducting wildlife surveillance in-country (Figure 1). The opportunity for capacity development reported most commonly as important by wildlife officials was enhancing communication and coordination among agencies, sectors, or regions (Figure 2). PREDICT project scientists in the 16 countries reported most frequently that increasing human and laboratory capacities and a heightening awareness of wildlife disease surveillance presented the most important opportunities for building capacity (*Schwind et al. 2014a*).

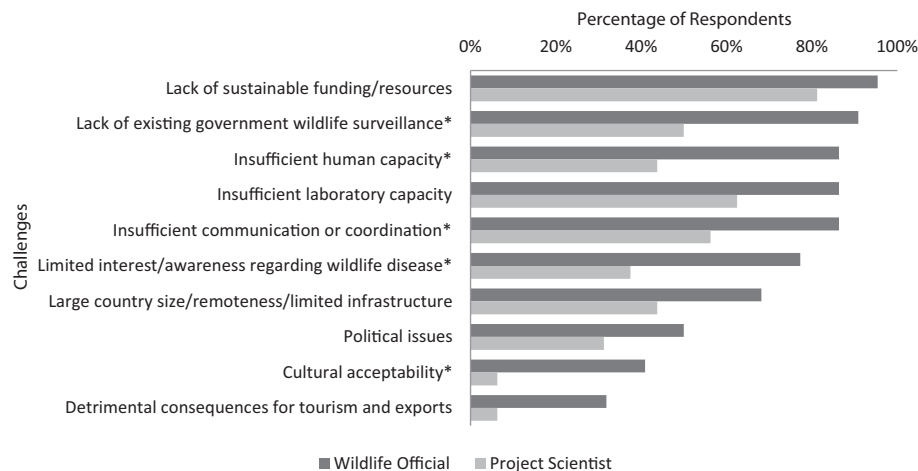


Figure 1. Challenges associated with conducting zoonotic pathogen surveillance ranked by wildlife officials and project scientists as ‘important’. *indicates a statistically significant difference ($p < 0.05$) between perspectives among two stakeholder groups. From Schwind et al. 2014a.

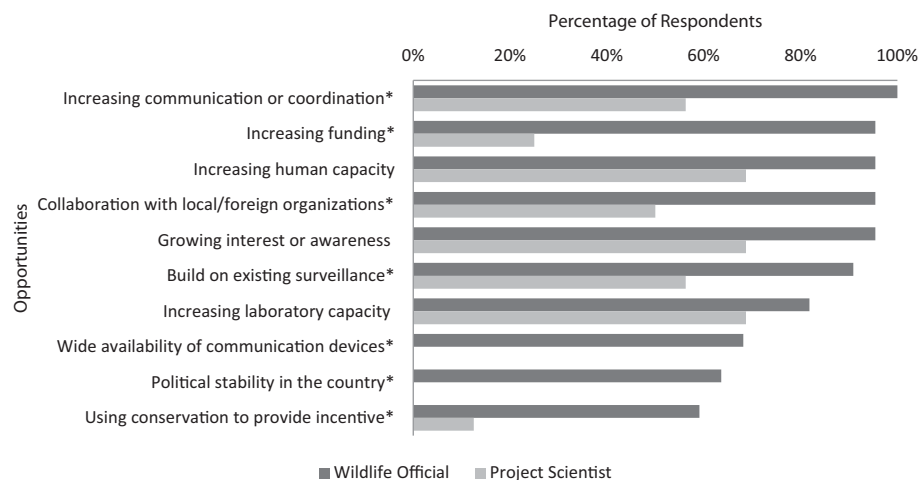


Figure 2. Opportunities associated with conducting zoonotic pathogen surveillance ranked by wildlife officials and PREDICT project scientists as ‘important’. *indicates a statistically significant difference ($p < 0.05$) between perspectives among two stakeholder groups. From Schwind et al. 2014a.

Surveillance Strategies to Maximize Detection of Zoonotic Viruses in Wildlife

PREDICT researchers produced a number of manuscripts that improve our understanding of the most ideal surveillance strategies for maximizing detection of zoonotic pathogens in wildlife. *Levinson et al. (2013)* conducted a systematic literature review to compare the zoonotic virus discovery potential between syndromic surveillance of diseased animals and active surveillance of apparently healthy animals. The authors constructed a database with information collected from the literature on mammal-virus associations and on whether each virus has been documented to cause disease in the wildlife host. The results provide evidence that infected bats and rodents are less likely to show clinical signs of disease compared to other mammalian taxa. In addition,

the findings reveal that a mixed surveillance strategy of sampling passively, actively reporting mortalities, and broad surveillance of healthy wildlife is generally ideal for virus discovery. The authors concluded that surveillance of apparently healthy wildlife will maximize zoonotic virus discovery potential, especially in bats and rodents. To improve efficiency, the authors also recommended focusing surveillance efforts in regional emerging disease hotspots and on wildlife taxa documented to carry the highest percentage of zoonotic pathogens (*Levinson et al. 2013*).

Using data generated from PREDICT surveillance activities, *Anthony et al. (2013a)* provided the first ever robust estimate of total diversity of known and novel viruses in a mammalian species and the sampling effort needed to detect a proportion of the viral richness. The authors utilized PREDICT viral discovery protocols on specimens from repeatedly sampled *Pteropus giganteus* fruit bats to saturate the discovery of new viruses (Figure 3). Ecological statistical approaches applied to the viral discovery results revealed that the total mammalian diversity of viruses is approximately 320,000. The costs to discover all 320,000, annualized over a 10-year study time frame, would represent a small fraction of the cost of many pandemic zoonoses and could allow spillover to be detected early and spread to be halted at the source (*Anthony et al. 2013a*).

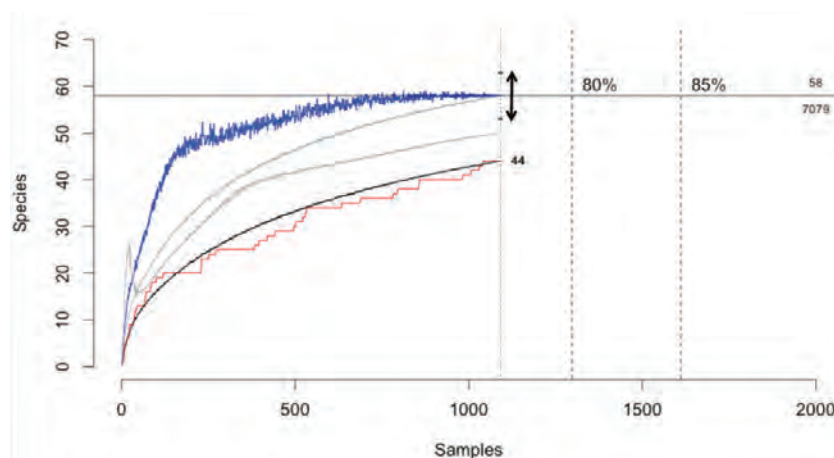


Figure 3. Number of samples examined versus the number of new species of viruses detected. Red line = actual number of new viruses found in each sample. The number of new viruses discovered in these samples is 44. Black line = estimated discovery curve. Blue line = Chao2 estimator. The Chao2 estimator is used to calculate the maximum number of new viruses. In this case, we expect to find 58 new species of viruses. In order to identify 100% of the new viruses, 7,079 samples would need to be tested. However, only a little more than 1,300 samples would be needed to identify 80% of the new species of viruses. This methodology informs on optimal surveillance strategies and sample size targets. Adapted from Anthony et al. 2013a.

In addition, PREDICT contributed to a study quantifying the prevalence of different viruses across bat species and various bat biological sample types using data obtained from a systematic search of the past 7 years of literature (*Weekley and Olival in review*). Information obtained from a review of 95 published studies on bat viral discovery was used to develop predictive models to assess which factors best explain success in viral discovery in bats.

Olival and Hayman (2014) reviewed the ecology and epidemiology of Marburgviruses and Ebolaviruses in bats and highlighted important areas to focus future research. The authors discuss what is known about the dynamics of filoviruses in bats (Figure 4) and drew comparisons with other bat-borne zoonoses. They also discussed advanced diagnostic methods used to detect

filovirus infection and exposure in bats and how these assays along with experimental studies and innovative statistical methods can inform on ecological studies necessary to elucidate viral persistence in wildlife populations and virus spillover into people. The authors highlighted how these studies are needed to identify potential mitigation strategies to reduce the risk of human exposure and advocated for a coordinated global surveillance strategy for filoviruses in wildlife (*Olival and Hayman 2014*).

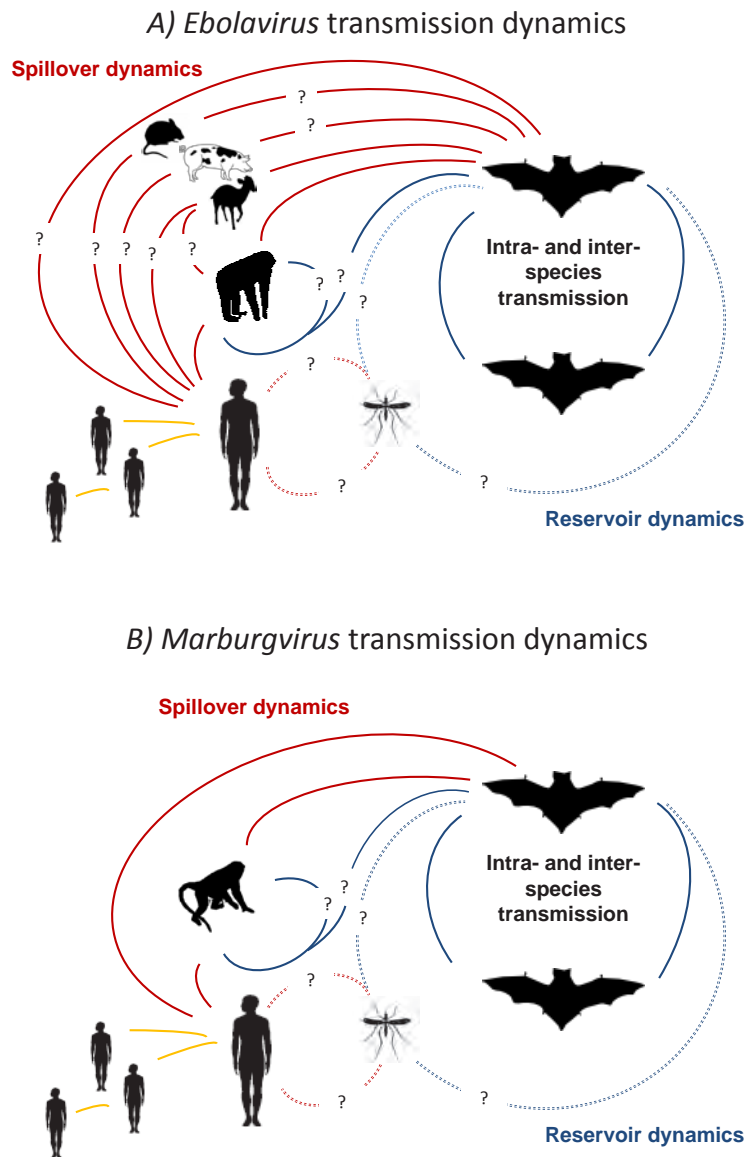


Figure 4. A) Multiple transmission pathways for *Ebolavirus* genera viruses. Potential reservoir dynamics are shown in blue, spillover epidemics in small mammals (Africa), pigs (Reston ebolavirus only), duikers (Africa); and B) Multiple transmission pathways for *Marburgvirus* genera viruses. Vectors for both genera are unlikely significant, but not known (dashed line). Those with epidemiological uncertainty are shown with question marks. Potential reservoir dynamics are shown in blue, spillover epidemics in primates and humans shown in red and ongoing human transmission in orange. From *Olival and Hayman 2014*.

PREDICT researchers also evaluated the most important routes or pathways of transmission for emerging zoonoses (*Loh et al. in review*) using information reported on 335 emerging infectious disease events in people from 1940 to 2004. The results revealed that the major transmission pathways for zoonoses differ according to the specific emerging infectious disease driver (i.e. factor or process influencing emergence) and could be used to better target surveillance and more effective control of newly emerging zoonoses in areas experiencing different underlying anthropogenic pressures. The authors discuss how a focus on transmission pathways for disease surveillance and control measures could improve cost-effectiveness because multiple pathogens circulating at a high-risk human-animal interface often share common transmission pathways (*Loh et al. in review*).

In addition, PREDICT scientists conducted research to optimize noninvasive sampling techniques for disease surveillance for wildlife taxa that are difficult to locate and capture (i.e. nonhuman primates). PREDICT assisted with development of the first successful noninvasive method to assess previous exposure to ebolaviruses in wild apes through detection of antibodies in feces (*Reed et al. 2014*). Ebola virus outbreaks in wild apes result in significant mortality and are thought to have caused recent declines in great ape populations in Africa. In addition, Ebola virus disease in people has been linked to contact with carcasses of infected wild animals, including nonhuman primates. This diagnostic technique, which identified antibodies in up to 10% of fecal samples deposited by gorillas, will improve early detection of outbreaks and will also aid in identifying immunologically naïve populations of wild apes that could benefit from protective interventions, such as immunization (*Reed et al. 2014*). The assay holds promise to detect antibodies against other pathogens in free-ranging nonhuman primates.

In coordination with this effort, *Olson et al. (2012b)* used simulation modeling to assess optimal sampling strategies for detecting fecal samples from gorillas in Central Africa. The authors simulated a number of different sampling survey designs to identify which design maximized the number of fecal samples detected while also producing accurate estimates of gorilla population densities. The designs were evaluated for accuracy and cost and time efficiencies over a variety of different gorilla population densities and distributions. A mixed sampling design combining traditional transect and directed reconnaissance maximized the detection of fecal samples and

estimates of gorilla density, while targeted reconnaissance sampling maximized sampling efficiency but produced biased population density estimates (*Olson et al. 2012b*).

PREDICT also provided recommendations on targeted pathogen surveillance in the wildlife trade. *Karesh et al. (2012b)* reviewed the risk of emerging infectious diseases from the trade of wildlife and wildlife products and the impact of the trade on biodiversity loss. The authors' recommendations included focusing efforts at markets and other critical points in the trade where cost-effective approaches could be implemented to decrease disease transmission risks in animals and people and also reduce threats to biodiversity (*Karesh et al. 2012b*).

Ebola virus is a major threat to lowland gorillas.



PHOTO BY MIKE CRANFIELD

Surveillance Strategies for Zoonotic Disease Outbreak Investigations

Our researchers also used data generated from PREDICT surveillance efforts and information obtained through systematic literature reviews to direct wildlife surveillance activities during human zoonotic disease outbreak investigations. For example, discovery of a betacoronavirus (b-CoV) with 96.5% amino acid identity to Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) in a *Nyctinomops laticaudatus* bat in Mexico by **Anthony et al. (2013b)** directed targeted surveillance of bats in Saudi Arabia during MERS outbreak investigations. Sampling led to the discovery of a MERS-CoV sequence in a Saudi Egyptian tomb bat that had 100% nucleotide identity to the virus from the human index case-patient (*Memish et al. 2013*).

PREDICT conducted a literature review to determine what is known regarding potential sources of Ebola virus spillover into humans and animals. The aim of the review was to provide evidence-based animal surveillance recommendations for a series of human Ebola virus disease outbreak investigations in Africa (**Olson et al. 2012a**). The authors provide information on susceptible target species and guidance on animal sampling for disease outbreaks occurring in resource-limited regions, including which diagnostic assays should be prioritized to most quickly detect Ebola virus in animal hosts. They also provided specific recommendations for targeted surveillance aimed at identifying potential sources of transmission from animals to humans including: 1) prioritize surveillance of free-ranging human and nonhuman primate mortality and morbidity events; 2) investigate all wildlife morbidity and mortality events; 3) sample carcasses (vs. live animals) for a higher likelihood of Ebola virus and virus RNA recovery; 4) prioritize dogs and pigs among domestic animals for sampling and screen samples for virus and antibodies; and 5) surveillance of bats when large sample sizes ($n > 100$) are feasible (**Olson et al. 2012a**).

Development of Quantitative Tools to Improve Efficiency of Surveillance

A number of other studies by PREDICT researchers have focused on strategies and tools to increase the efficiency of surveillance efforts in resource-limited settings. **Bogich et al. (2013)** developed a tool to enhance assessment in the early stages of a disease outbreak in settings with limited resources and capacity for surveillance and diagnosis. The authors constructed a network model to evaluate clustering of outbreaks of the same disease and also of different diseases with regard to seasonality, case fatality ratio, and symptoms. Outbreaks of undiagnosed encephalitis in South Asia were selected from ProMED-mail data to test in the model. The results indicated that Nipah virus could be identified very early on during an outbreak, without laboratory results, using simple data reported in real-time (**Bogich et al. 2013**).

In addition, through the HealthMap platform, PREDICT and partners at HealthMap introduced an open source automated web crawling surveillance method designed to monitor official and unofficial reports of illegally traded wildlife and wildlife products (**Sonricker Hansen et al. 2012**).

The MERS-CoV sequence was detected in a sample from an Egyptian tomb bat, *Taphozous perforatus*, that was captured in an area with MERS cases in Saudi Arabia.



PHOTO BY ECOHEALTH ALLIANCE

Unfortunately, due to its clandestine nature, no comprehensive database exists on the scope, scale, and extent of the wildlife trade. Building on tools developed for early disease detection using the internet, PREDICT and HealthMap created a site (healthmap.org/wildlifetrade) that collects information from global digital media on wildlife trade and presents illegal wildlife trade reports worldwide in near real-time using an interactive visualization display. Drawing on unofficial sources, such as online news sites and social media, to obtain information on the illegal wildlife trade offers a novel approach to supplement information collected through official reporting (*Sonricker Hansen et al. 2012*). Increased monitoring of the wildlife trade can offer greater insight into the pathways promoting disease emergence within this high-risk human-wildlife contact interface.

PREDICT also launched a local media surveillance (LMS) pilot study in seven countries to monitor disease events reported in local media sources and assess the value and utility for LMS to enhance digital surveillance and early recognition of disease events (Figure 5; *Schwind et al. 2014b*). Over a 16 week evaluation period, the system was evaluated on a number of attributes including simplicity, timeliness, and acceptability. The investigators found that LMS filled critical gaps in global disease recognition and monitoring by contributing local information to HealthMap. In addition to providing useful and timely information on disease events at the local level, the LMS was easy to implement and required minimal resource commitment. Another advantage of LMS was the diverse languages supported, in contrast to digital media surveillance platforms that do not currently support all languages.



Figure 5. Data and information flow through the local media surveillance pilot. From Schwind et al. 2014.

Lastly, *Funk et al. (2013)* developed the first ever analysis of case data for a series of important diseases normally considered emerging, to test whether they were truly emerging or not. Segmented regression was used to test whether cases were rising significantly (emerging), stable (emerged), or declining ('receding' diseases). The findings show that many diseases that are considered emerging are endemic. The authors proposed ways to use this tool to reallocate resources for surveillance, control, and prevention (*Funk et al. 2013*).

RISK CHARACTERIZATION

PREDICT conducted a number of studies to assess risk associated with landscape level drivers and specific human behaviors perceived to increase the probability of spillover and spread of zoonotic diseases in people.

Assessment of Factors Influencing Zoonotic Virus Emergence and Spread

Murray and Daszak (2013) reviewed key mechanisms for novel zoonotic disease emergence due to land-use change and provided approaches for testing two hypotheses on how land-use change leads to emergence of zoonotic viruses in humans (Figure 6): 1) land-use change perturbs natural ecological systems, which disrupts natural pathogen dynamics in wildlife and promotes cross-species transmission (including spillover to humans and domestic animals); and 2) increases the rate or type of contact between hosts (including humans) and novel pathogens, which may also increase spillover risk. The authors provided the framework for the theory, hypotheses, and focus of the Deep Forest study, a PREDICT project intended to improve our understanding of how land-use change affects opportunities for cross-species transmission of zoonotic pathogens through an investigation of pathogen diversity across different degrees of land development (i.e. urban, peri-urban, and undeveloped; *Murray and Daszak 2013*).

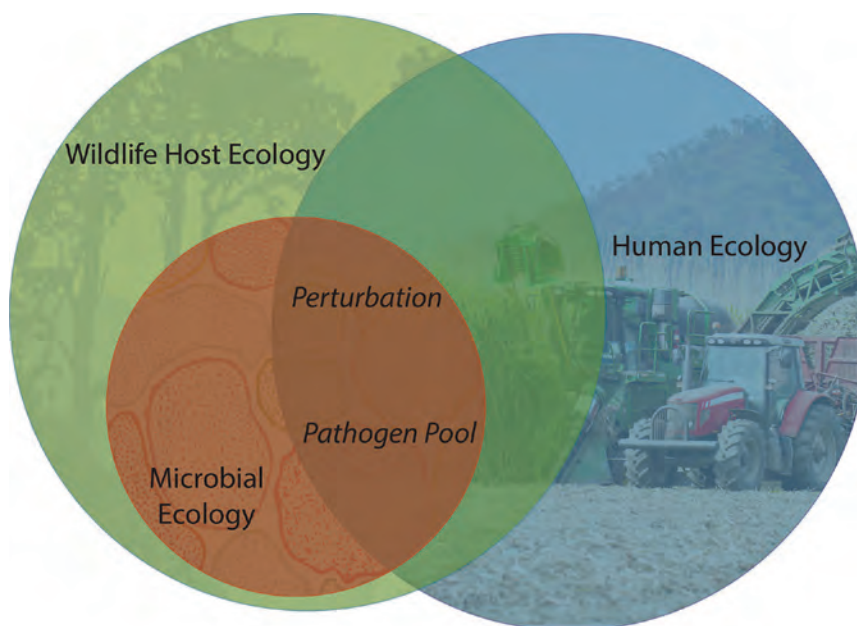


Figure 6. Conceptual model of how land-use change drives the emergence of infectious diseases in people. The authors propose two not mutually exclusive hypotheses: 1) anthropogenic activities in previously pristine environments bring people into contact with a large reservoir of microbial diversity in wildlife for which humans are naïve ('pathogen pool hypothesis'); and 2) land-use changes alter the dynamics of pathogen transmission among wildlife and promote cross-species transmission ('perturbation' hypothesis). From *Murray and Daszak 2013*.

Loh et al. (2013) discussed the value of applying ecological theory and methodology to investigations of the dynamics of zoonotic diseases. The authors reviewed methodologies for studying wildlife diseases and presented a number of ecological approaches and discussed their

potential applications to inform on disease prevalence and dynamics. They also emphasized the need for a greater focus on ecology in the One Health approach to improve our understanding of zoonotic disease dynamics and risk and how this will be particularly important in the future, as increasing anthropogenic change leads to greater opportunities for disease emergence (**Loh et al. 2013**). Similarly, **Preston et al. (2013)** reviewed ecological concepts, such as networks of population dynamics, community structure, and ecosystem matrices and their relevance to health. The authors described a network context for EcoHealth research, providing a conceptual framework for ecosystem structure and disease emergence.

Rostal et al. (2013) developed a tool to use co-phylogeny of hosts and their pathogens to predict the likelihood of spillover of zoonotic pathogens from wildlife hosts to people. This manuscript describes the important role of wildlife as environmental indicators and demonstrates the need to understand the critical linkages between humans and wildlife through targeted surveillance and research (**Rostal et al. 2013**). Similarly, **Lei and Olival (2014)** used bats and rodents and *Bartonella* spp. and *Leptospira* spp. as a model to develop a tool to use co-phylogeny of hosts and their pathogens to predict likelihood of spillover. This tool can be broadly applied to viruses and other emerging diseases.

In addition, PREDICT researchers augmented previous analyses of “EID hotspots” with additional information on drivers of emerging disease, including a global model to identify the drivers of bat-borne zoonoses (**Brierley et al. in review**). An outcome of this research will be the first EID hotspot map for zoonoses originating from bats.

In order to make recommendations for pandemic prevention, **Bogich et al. (2012b)** assessed drivers of (i.e. factors influencing) outbreaks of international concern. The distributions of disease outbreaks were assessed across driving factors using a database containing information from approximately 400 outbreaks. The most important driver was a breakdown or lack of public health infrastructure (Figure 7). While not unexpected, these results have not previously been presented using real data on a global scale. The authors recommended a proactive systems approach by which international development organizations prioritize mainstream development funds versus emergency response funds to address pandemic prevention (**Bogich et al. 2012b**).

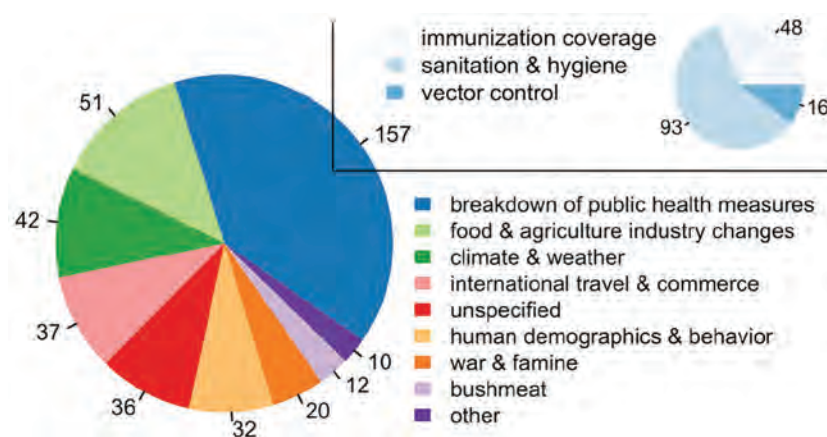


Figure 7. The number of outbreaks by driver. The subplot shows the subdrivers within the category “breakdown of public health measures”. From Bogich et al. 2012b.

Assessment of the Effect of Climate Change on Viral Emergence

PREDICT researchers also illustrated how ecological niche models for specific pathogens may be utilized to assess the potential effect of climate change on the occurrence and spread of zoonoses. For example, *Daszak et al. (2013)* used ecological niche modeling to estimate where the spatial distribution of *Henipavirus* hosts is likely to expand, contract, or remain the same. Results indicate that the spatial distribution of *Henipavirus* reservoir hosts, and therefore henipaviruses, will likely vary under climate change scenarios, potentially leading to expanded areas for emergence in people. This modeling framework has utility for assessing changes in spatial distributions of wildlife hosts with climate change and can inform on short-term and long-term surveillance strategies and management actions aimed at minimizing the risk of diseases emergence (*Daszak et al. 2013*).

In addition, *Thomassen et al. (2013)* used ecological niche analyses in combination with data on climate and remote-sensing to improve understanding of the distributions of monkeypox virus under present and future climate conditions. Models developed by the researchers are useful for evaluating where environmental conditions may become more suitable for human monkeypox and could assist with prioritization of regions for future monkeypox surveillance efforts. For example, results show that forest clearing and climate may act synergistically to increase the transmission of monkeypox virus from wildlife to humans and predict increased suitability for monkeypox virus in eastern Democratic Republic of Congo. In addition, these models can assist with prioritization of species to sample for surveillance. For example, they affirm previous findings that rope squirrels (*Funisciurus* spp.) may be important reservoirs and that monkeys (*Cercopithecus* spp.) as well as pangolin (*Manis tetradactyla*) should be investigated as potential reservoir species (*Thomassen et al. 2013*).

PATHOGEN DISCOVERY AND CHARACTERIZATION

PREDICT made major contributions to the detection and characterization of known and novel viruses (and other potential pathogens), circulating in wildlife.

Virus Discovery and Characterization in Bats and Rodents

The realization that bats host a wide range of viral zoonoses, including Nipah, Hendra, rabies, Ebola, Marburg, and SARS-like coronaviruses (CoVs) emphasizes the importance of surveillance for viruses in high risk wildlife taxa that have the potential to spillover into people. PREDICT conducted a number of viral discovery efforts in bats to enhance our understanding of the potential pool of viruses that pose a public health threat. For example, *Anthony et al. (2013b)* described the detection of 12 novel coronaviruses and one known coronavirus (9 alpha-CoVs and 4 beta-CoVs) from bats of 42 species sampled in Mexico. Phylogenetic analysis revealed that speciation of hosts is a strong selective driver in CoV evolution, even in related populations separated by significant geographical distance. The authors identified a beta-CoV with 96.5% amino acid identity to MERS-CoV in a *Nyctinomops laticaudatus* bat. Further studies to sequence the receptor binding domain are underway to evaluate whether this Mexican MERS-like CoV can bind to human cell surface receptors and therefore directly infect humans.

In addition, PREDICT sampled bats for Dengue virus (DENV) in pristine areas in the Calakmul (Campeche) and Montes Azules (Chiapas) Biosphere Reserves in southern Mexico and also landscapes in Mexico that had undergone anthropogenic changes. Six bats (4.1%) tested positive for Dengue serotype 2: four bats in Calakmul (two *Glossophaga soricina* bats, one *Artibeus*

jamaicensis bat, and one *A. lituratus* bat) and two bats in Montes Azules (both *A. lituratus*; **Sotomayor-Bonilla et al. 2014**). No effect of anthropogenic disturbance on the presence of DENV in bats was detected in this study; however, the species of bats exhibiting positive results are abundant and well-adapted to disturbed habitats in the Neotropics.

Based on the discovery of the virus closely related to MERS-CoV in the Mexican bat, staff with the support of the Saudi Ministry of Health, conducted targeted surveillance of chosen bat species in regions of Saudi Arabia where MERS cases had been previously identified (Memish et al. 2013). A variety of CoVs were detected, including a virus sequence in a *Taphozous perforates* bat that had 100% nucleotide identity to the virus found in the index case, suggesting that bats may play a role in human infection (Memish et al. 2013). PREDICT researchers also detected a MERS-like CoV in a bat in Thailand (**Wacharapluesadee et al. 2013**). The authors assessed the risk of exposure of bat guano miners to coronaviruses, Nipah virus, and *Histoplasma capsulatum* from guano harvested for sale as agricultural fertilizer. Samples were positive for coronaviruses, including the MERS-like CoV. Follow-up studies are underway to assess risk of spillover to the guano miners.

PREDICT conducted the first isolation and characterization of a SARS-like coronavirus from a Chinese horseshoe bat (*Rhinolophus sinicus*; **Ge et al. 2013**). The virus was found to bind to the human ACE-2 cell receptor (Figure 8), suggesting that direct transmission to humans from bats is possible. Previously, the only known source of SARS for humans was civets sold in markets. During the 2003 outbreak of SARS in the wet markets of Guangdong province in China, it was thought that bat viruses first infected civets and then evolved to infect people through this intermediate host. However, this study provides compelling evidence that an intermediate host was not necessary (**Ge et al. 2013**). Isolation of the live SARS-like virus from bats will allow for future studies to identify potential effective control measures, including vaccine development.

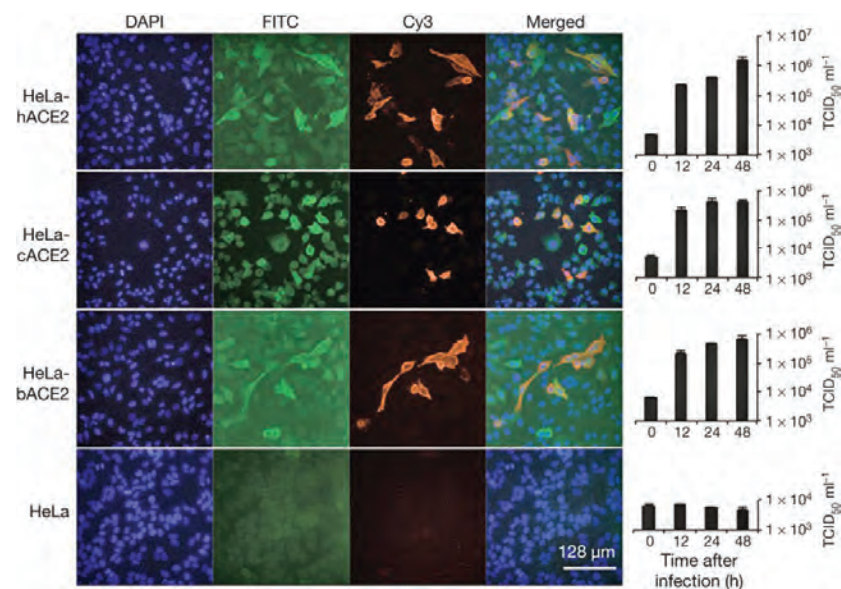


Figure 8. Visualization of virus infectivity in cell culture with and without the expression of ACE2. b, bat; c, civet; h, human. From left to right, the columns show staining of nuclei (blue), ACE2 expression (green), virus replication (red), merged triple-stained images, and real-time PCR results ($n = 3$). The error bars represent the standard deviation. From Ge et al. 2013.

PREDICT researchers also led a number of other studies to investigate the diversity of viruses in bats. **Epstein et al. (2010)** used high-throughput pyrosequencing to investigate novel viruses in serum samples obtained from frugivorous bats (*Pteropus giganteus*), the reservoir for Nipah virus in Bangladesh. A novel flavivirus related to Hepatitis C and GB viruses was detected. Sequencing results suggest *P. giganteus* is a natural reservoir. This study expands our knowledge of the wildlife host range for GB-like viruses and illustrates the value of unbiased sequencing as a tool for virus discovery. PREDICT researchers also used unbiased high-throughput sequencing to uncover a highly diverse group of bat-derived viruses in African and Central American bats that are related to hepaciviruses and pegiviruses within the family Flaviviridae (**Quan et al. 2013**). Additional PCR screening of bat samples collected worldwide detected these viruses in North America and Asia. Evolutionary analyses indicated that all known hepaciviruses and pegiviruses, including those from humans and other primates, originate in bats. The diversity, phylogenetic divergence, and global distribution of the viruses provide evidence that bats are a natural reservoir for hepaciviruses and pegiviruses and further enhance our understanding of the evolutionary history of hepatitis C virus and the human GB viruses (**Quan et al. 2013**).

In addition, PREDICT used reverse transcription polymerase chain reaction (RT-PCR) assays and next-generation sequencing to characterize viruses detected in the feces of 20 common species of insectivorous and frugivorous bats (281 individual bats) in Yunnan province in China (**Yuan et al. 2014**). Paramyxoviruses were detected in seven bats using RT-PCR, and these paramyxoviruses were mainly classified into three genera (*Rubulavirus*, *Henipavirus*, and *Jeilongvirus*). Additional novel viruses were detected in the paramyxovirus-positive bats using next-generation sequencing. Overall, the most frequently identified viruses, particularly in bats from the family *Hipposideridae*, were retroviruses (**Yuan et al. 2014**). This research expands our understanding of the bat virome in species commonly found around Yunnan, China, and provides insight into the diversity of viruses that may be capable of spilling over into people.

In another PREDICT study, **Weiss et al. (2012)** investigated the risk of cross species transmission of paramyxoviruses (i.e. respirovirus, morbillivirus, and henipavirus) associated with hunting, preparation, and consumption of bats in the Republic of Congo. Nipah and Hendra viruses are highly pathogenic paramyxoviruses that cause acute respiratory illness and encephalitis in people, and greater than 90% of infected individuals die (Luby and Gurley 2012; Marsh and Wang 2012). Samples obtained from live straw-colored fruit bats (*Eidolon helvum*) captured by hunters for bushmeat were screened for paramyxoviruses by PCR. Viral sequences from 11 bats formed at least three distinct groups in the *Paramyxoviridae* family (**Weiss et al. 2012**). Henipaviruses cluster in-between the *E. helvum* paramyxovirus sequences. Phylogenetic analysis revealed no spatial distinction between the sequences in *E. helvum* in the Republic of Congo and previous sequences detected in bats from Ghana, suggesting that various strains are exchanged over large distances by the migrating bats (**Weiss et al. 2012**). Transmission of henipaviruses from fruit bats to humans has been documented in Australia and Asia (Luby and Gurley 2012; Marsh and Wang 2012). To determine the potential for henipavirus spillover to people in Africa, PREDICT screened hunted fruit bats and people living in villages in Cameroon for antibodies to henipaviruses using a highly specific serological assay (**Pernet et al. in press**). Approximately 48% and 3-4% of the bats and humans, respectively, were seropositive. The most significant risk factors for seropositivity in people were butchering bats for bushmeat and living in areas impacted by deforestation (**Pernet et al. in press**).

Olival et al. (2013b) used serology and PCR to assess whether there was evidence of Ebola virus infection in bats in Bangladesh. Serum samples were tested for antibodies against both Reston

ebolavirus and Zaire ebolavirus. A number of seropositive samples were identified in fruit bats (i.e. *Rhinolophus leschenaulti*, *Cynopterus* spp., and *Miniopterus lyra*), and the positive sera reacted more strongly to Zaire ebolavirus antigens than Reston ebolavirus antigens. These findings suggest exposure to an Ebola virus distinct from Reston ebolavirus, the only filovirus documented to date in Asia. This study extends our knowledge of the range of Zaire ebolavirus, or some related strain, to mainland Asia (*Olival et al. 2013b*).

PREDICT researchers also investigated the genetic diversity of astroviruses in a wide range of small mammalian species, most of which were rodents and bats, in order to gain insight into the ecology and evolution of these viruses (*Hu et al. 2014*). Astroviruses have a worldwide distribution and are one of the primary pathogens causing viral gastroenteritis in children (Moser and Schultz-Cherry 2005). The researchers detected known and novel astroviruses in bats and novel astrovirus groups in rodents, shrews, and pikas. Molecular analyses revealed a close phylogenetic relationship between some of the rodent and ungulate astroviruses suggesting that cross-species transmission could occur (*Hu et al. 2014*).

Furthermore, PREDICT contributed to research exploring factors associated with microbial (viral, bacterial, and parasitic) species richness in bats sampled in Southeast Asia (*Gay et al. 2014*). The results highlighted the key role of bat distribution shape on parasite diversity. Specifically, bat species living in large colonies and fragmented distributions have lower viral species richness relative to bat species living in small colonies and continuous distributions. This study is an important first step in understanding factors driving microbial species richness in bats.

Virus Discovery and Characterization and Cross-species Transmission in Nonhuman Primates

Our researchers conducted a number of studies on pathogen discovery and characterization in settings where there is a high risk of cross species transmission between people and nonhuman primates. Hunting and butchering of wild animals provides nutrition and livelihoods in many countries across Africa. In addition to hunting and consumption by locals, there is also an increasing regional and global market for bushmeat, including hunted nonhuman primates. These practices lead to increased risk of spillover of nonhuman primate-origin pathogens into people (Wolfe 2005;

Woolhouse 2002; Ahuka-Mundeye et al. 2011; Peeters et al. 2002; and Smith et al. 2012).

Human immunodeficiency viruses types 1 and 2 provide examples of pandemic infections in humans resulting from cross-species transmission from simian immunodeficiency virus (SIV) infected nonhuman primates (*Sharp et al. 2010*). The documented spillover into people and pandemic potential of simian retroviruses illustrate the potential public health threat associated with these viruses (*Switzer et al. 2012*).

Prior to initiation of the Emerging Pandemic Threats program, our scientists were investigating the risk of emergence of simian retroviruses in people with close interactions



PHOTO BY PAUL T. TELFER

with primates in central Africa. These researchers built upon this previous work by contributing to a number of studies on this topic. PREDICT contributed to research that documented simian foamy virus, a simian retrovirus, infection in primate hunters in Cameroon and Gabon. The researchers screened a large population of rural Democratic Republic of Congo (DRC) inhabitants for simian foamy virus infection and investigated risk factors for exposure (*Switzer et al. 2012*). Sequence analysis revealed human infection with new simian foamy viruses from Angolan colobus and red tailed monkeys, two nonhuman primate species hunted frequently in DRC. Unlike previous studies, women were not at lower risk of infection with simian foamy viruses relative to men in this study (*Switzer et al. 2012*). Men are the primary hunters in DRC, while women more commonly perform the food preparation, suggesting that exposure may be as or more frequent with bushmeat preparation compared to hunting.

LeBreton et al. (2014) screened blood samples collected from 21 different species of nonhuman primates in Cameroon for simian T-lymphotropic virus type 4 (STLV-4) to discover how human T-lymphotropic virus type 4 (HTLV-4) emerged and to gain insight into STLV-4 evolution and pandemic potential. Among the species of nonhuman primates tested, only gorillas had positive results for STLV-4. The diversity of nonhuman primate species tested and detailed phylogenetic analyses performed for this study indicated that gorillas are the nonhuman primate reservoir of HTLV-4 and that interspecies transmission likely occurs through the hunting and butchering of wild gorillas (**LeBreton et al. 2014**). This discovery illustrates the diversity of retroviruses transmitted to people via the same pathway used by HIV in its emergence. In addition, **Djoko et al. (2012)** assessed SIV transmission to humans by evaluating infection with SIV in individuals who hunt and butcher nonhuman primates in Cameroon, a population in which simian foamy virus and simian T-lymphotropic virus were previously detected. Seroreactivity to SIV was detected in 23 individuals; however, nucleic acid sequences of SIV genes could not be detected, suggesting that SIV infection in humans could occur at a lower frequency than with other simian retroviruses (**Djoko et al. 2012**).

PREDICT characterized a new simian immunodeficiency virus strain in a naturally infected *Pan troglodytes* chimpanzee with AIDS-related symptoms. **Etienne et al. (2011)** described the clinical history and viral evolution of a naturally simian immunodeficiency virus SIV infected *P. t. troglodytes* chimpanzee, the reservoir of the ancestors of HIV-1 in humans. Results of this study show that SIV has an increasing viral diversity over time and suggest that *P. t. troglodytes* chimpanzees can also have clinical progression to an AIDS-like disease, as has been previously described in *P. t. schweinfurthii*, despite the broadly held belief that SIVs do not cause pathology in their natural hosts (**Etienne et al. 2011**).

Lyons et al. (2012a) investigated species specificity and potential for cross-species transmission of hepatitis B virus (HBV) among nonhuman primate species and between humans and nonhuman primates using complete genome sequencing and phylogenetic analysis. Results of this study provide the first evidence for HBV circulation between chimpanzees and gorillas and among subspecies of chimpanzees, a conclusion that differs from the dogma of strict host specificity of HBV genotypes and suggests there is potential for spillover (and the emergence of new genotypes) of this virus into new species, including humans (**Lyons et al. 2012a**).

Similarly, **Harvala et al. (2012)** documented evidence for the potential for cross-species transmission of enteroviruses between people and nonhuman primates. The authors compared serotype-specific neutralizing antibodies against three enterovirus types in chimpanzees, gorillas, and old world monkeys. Enterovirus species A, B, and D were detected in wild chimpanzees,

demonstrating their potential widespread circulation in nonhuman primates. These findings contribute to our understanding of the host range of enteroviruses, which have been considered to be primarily human viruses. The potential for spillover and spread of enteroviruses from old world monkeys or apes to humans is unknown; however, the worldwide outbreak of EV-D70 that initially spread from an area in central Africa (Kono et al. 1972; Kew et al. 1983) suggests that it may be plausible. Evidence of enterovirus circulation among apes and old world monkeys warrants reassessment of these nonhuman primates as potential sources for the periodic emergence of novel enterovirus types into previously unexposed human populations (*Harvala et al. 2012*).

Sharp et al. (2010) provided the first evidence for the existence and widespread infection of Cameroonian chimpanzees and gorillas with parvoviruses antigenically related to PARV4, human bocavirus (HBoV), and B19 virus in humans. To further investigate whether interspecies transmission is possible for primate PARV4-like viruses, *Adlböck et al. (2012)* investigated the species specificity of PARV4-like viruses in nonhuman primates (i.e. chimpanzees and their prey of colobus monkeys) and humans who hunt primates in a setting in Cameroon where transmission of simian viruses has occurred between chimpanzees and colobus monkeys (Leendertz et al. 2004; Leendertz et al. 2008). PARV4-like virus infection is widespread in chimpanzees, colobus monkeys, and humans in West Africa; however, the PARV4-like viruses appear to be species specific, despite circumstances that present vast opportunities for cross-species transmission. These findings suggest that the risk of spillover of PARV4-like viruses from nonhuman primates into humans in West Africa is low (*Adlböck et al. 2012*). PREDICT researchers also detected sapoviruses in nonhuman primates living in close contact with people in the Republic of Congo. Sapoviruses are viruses within the Caliciviridae family that cause gastroenteritis in people. Unbiased deep sequencing of the entire genomes revealed clustering within the human GI clade suggesting that cross-species transmission could occur (*Mombo et al. 2014*).

Investigation into a respiratory outbreak in mountain gorillas in Rwanda by PREDICT researchers revealed human metapneumovirus infection in affected individuals (*Palacios et al. 2011*). The source of the virus is unknown; however, the strain was most recently described in South Africa and likely was transmitted to the gorillas by humans, illustrating the potential

for bi-directional spillover of pathogens (*Palacios et al. 2011*). The parks where mountain gorillas reside are surrounded by very dense human populations, and research and ecotourism bring thousands of people in direct and indirect contact with the gorillas. The majority of wild mountain gorillas are habituated to the presence of humans for ecotourism, which is an important source of revenue for park management. The frequency and severity of respiratory disease outbreaks among mountain gorillas in the Virunga Massif have increased. *Spelman et al. (2013)* documented 18 outbreaks of respiratory disease among these gorillas between 1990 and 2010. Clinical signs, response to treatment in medically managed



PHOTO COURTESY OF TODD KELLY

cases, and post-mortem examination findings were consistent with upper respiratory infections with a primary viral cause that was in some cases accompanied by a secondary bacterial infection (Spelman *et al.* 2013). As a result of the increasing incidence of infections, the rules surrounding ecotourism visitation were designed to minimize the risk of disease transmission between visitors and wild human-habituated gorillas.

Gorillas are poached for the wildlife trade and confiscated orphaned gorillas are placed into captivity where they are provided constant contact and care by staff. In Rwanda, an orphaned Grauer's gorilla confiscated from poachers and held in captivity for more than two years developed oral lesions (Figure 9). Human herpes simplex virus Type 1 (HSV-1) was detected in this gorilla (Figure 10; *Gilardi et al.* 2014). This study conducted by PREDICT and its partners was the first to document spillover of viruses from humans into gorillas that are in close and frequent contact with people and provide further evidence of the bi-directional spillover of pathogens between people and non-human primates (*Gilardi et al.* 2014).



Figure 9. Vesicular stomatitis caused by HSV-1 in a juvenile Grauer's gorilla (*Gorilla beringei graueri*) confiscated from poachers in Goma, Democratic Republic of Congo. From *Gilardi et al.* 2014.

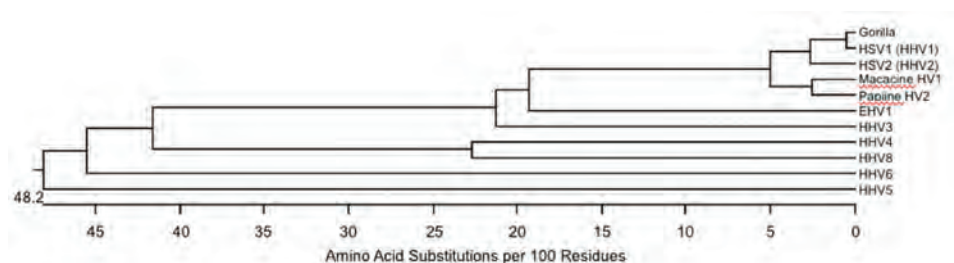


Figure 10. Phylogenetic analysis of the nucleotide sequence from the swab sample amplicon from the gorilla with the corresponding regions of HSV-1. From *Gilardi et al.* 2014.

Kading et al. (2013) documented exposure to zoonotic arboviruses in a number of African wildlife species, including mandrills. This study provides evidence that mandrills in Gabon and duikers in the Congo are possible reservoir species for arbovirus circulation, as prevalences of specific neutralizing antibodies were very high in these animals prior to documented arbovirus outbreaks in humans (*Kading et al. 2013*). Many zoonotic arboviruses, including West Nile virus (WNV), chikungunya virus (CHIKV), Zika virus, and Usutu virus have become globalized and caused significant human morbidity and mortality worldwide since their discoveries in East Africa (*Kading et al. 2013*).

Lastly, PREDICT researchers also participated in a study investigating the origins of the malaria parasite, *Plasmodium vivax*. Results of this research indicate that gorillas and chimpanzees in central Africa are infected with malaria parasites that are closely related to *P. vivax* in people. The authors show that *P. vivax* is of African, not Asian origin, and that all extant human parasites evolved from a single ancestor that spread out of Africa (*Liu et al. 2014*). Also, the high prevalence of *P. vivax* in wild-living apes indicates the existence of a substantial zoonotic reservoir, which has implications for malaria eradication efforts (*Liu et al. 2014*).

Pathogen Characterization for Unusual Cases of Disease in Humans and Disease Outbreaks

PREDICT has also contributed to a number of investigations into human illness and disease outbreaks through pathogen detection and characterization. *Grard et al. (2012)* developed and optimized a new and highly sensitive technique to reconstruct the genome of a novel rhabdovirus

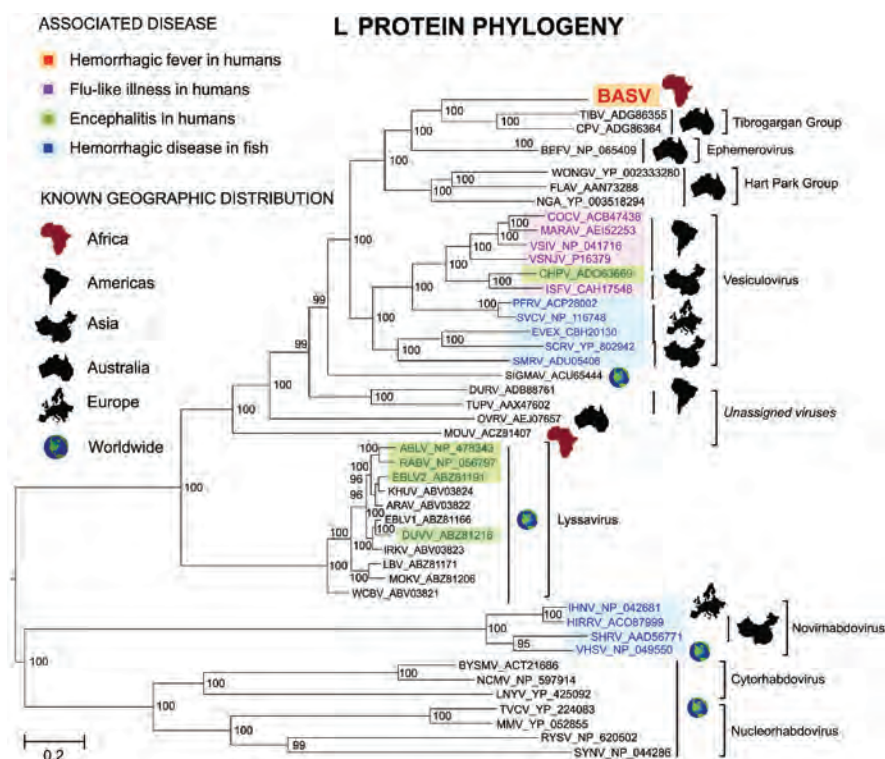


Figure 11. Phylogenetic analysis of the Bas-Congo virus and other rhabdovirus L proteins. The geographic distributions are indicated with specific icons and clinical syndromes associated with infection are indicated by unique color schemes. From Grard et al. 2012.

(Bas-Congo virus, or BASV) associated with three human cases of acute hemorrhagic fever in DRC in 2009 (Figure 11). Phylogenetic analysis revealed that BASV is highly divergent from other rhabdoviruses. The rhabdovirus family has never been documented to cause hemorrhagic fever in people and, because of this work, is now within the panel of viruses investigated in viral hemorrhagic fever outbreaks (*Grard et al. 2012*). Genetic analysis suggests an arthropod as a potential source, providing information for future investigations into potential reservoirs and modes of transmission. Further characterization of the virus glycoprotein showed that this virus may be infectious to a range of species (*Steffen et al. 2013*). BASV is potentially a novel BSL4 virus representing a credible threat to public health.

In addition, *Grard et al. (2011a)* characterized the Zaire ebolaviruses from the 2007 and 2008 outbreaks in Luebo, DRC. The authors found that the two Luebo ebolaviruses are similar to each other but are distinct from previous ebolaviruses characterized in DRC and in the Gabon-Republic of Congo area (*Grard et al. 2011a*). These findings strongly suggest that many Ebola outbreaks do not result from viral spread from previously identified foci, but from an independent viral emergence and that local wildlife populations (most likely bats) become infected and allow local viral persistence and reemergence from year to year.

Grard et al. (2011b) also investigated an isolated human case of Crimean-Congo Hemorrhagic Fever (CCHF) in the DRC in 2008. The authors used phylogenetic analysis to assess whether the virus resulted from a regional re-emergence or from the introduction of a novel virus in the area. The results suggest long-term ongoing CCHF virus circulation in Central Africa despite the absence of reported human cases (*Grard et al. 2011b*). Sporadic reporting of human cases could be partly associated with a specific sylvatic cycle in Central Africa where deforestation may increase the risk of re-emergence.

In addition, PREDICT evaluated the genomic diversity of monkeypox viruses in people with primary and secondary cases of infection (*Kugelman et al. 2014*). Phylogenetic analyses revealed four distinct lineages and a mutation that resulted in gene loss and that was associated with human-to-human transmission. The results suggest frequent spillover of the viruses from animals to people and potential increased adaptation to humans (*Kugelman et al. 2014*).

PREDICT team members also assisted with the investigation of the MERS outbreak in the Middle East. *Alagaili et al. (2014)* investigated the prevalence of MERS coronavirus infection in dromedary camels and other livestock in Saudi Arabia. The authors provide evidence that MERS coronaviruses have been circulating in dromedary camels for at least two decades, are widely distributed throughout the Kingdom of Saudi Arabia, and can be phylogenetically classified into clades with sequences of viruses recovered during outbreaks of MERS in people. No evidence of infection was found in other livestock (*Alagaili et al. 2014*). In addition, PREDICT researchers assisted with full-genome sequencing of MERS coronaviruses from nasal swabs of dromedary camels sampled in the Kingdom of Saudi Arabia which revealed that the MERS coronavirus sequences from dromedaries and humans are indistinguishable and that dromedaries can have co-infection with multiple genetic variants of MERS coronaviruses (*Briese et al. 2014*). Together, these findings provide evidence that dromedaries play a role in human infection.

In addition, PREDICT contributed to the characterization of the viruses causing the 2010 Chikungunya virus outbreaks in Yangjiang and Dongguang in China. Phylogenetic analysis revealed that the viruses had the closest relationship with the Singapore 2008 isolate belonging to the Indian Ocean clade and that the two outbreaks originated from different sources (*Wu et al. 2013*).

A molecular epidemiological survey of hepatitis C virus and pegivirus infection in people in the Democratic Republic of Congo (DRC) was conducted with the assistance of PREDICT researchers. The frequency of the lineages found in the DRC varied by age, suggesting different sources or events may explain the variation among different age groups of infected individuals (*Iles et al. 2013*). In a follow-up study, PREDICT researchers assisted with additional screening for hepatic C viruses in a larger number of blood samples collected from people throughout DRC (*Iles et al. 2014*). The sequences for hepatitis C genotype 4 viruses were combined with published sequences. The findings revealed that hepatitis genotype 4 originated in central Africa and that multiple lineages that cause human disease in Egypt arrived there from central Africa (*Iles et al. 2014*).

PREDICT also contributed to a capacity building effort to diagnose cases of acute febrile illnesses in Indonesia (*Myint et al. 2014*). Among patients enrolled in the study, one individual from Java who was sampled in 2004-2005 tested positive for West Nile virus (WNV; *Myint et al. 2014*). This study highlights the need for an efficient diagnostic test and enhanced surveillance and monitoring for WNV in Indonesia.

In addition, *Mokili et al. (2013)* discovered a highly divergent novel human papillomavirus by metagenomic analysis of samples from patients with febrile respiratory illness as part of a larger viral discovery effort using syndromic surveillance to investigate pathogens in military recruits from training facilities throughout the United States, residents of the US/Mexico border in and near San Diego, and military dependents. Additional viral discovery and characterization efforts utilizing archived samples from human patients presenting with fever or encephalitis of unknown origin are underway.

Virus Discovery and Characterization in Rodents and Hunted Wildlife in Latin America

Razuri et al. (2014) investigated hantavirus infection in rodents in a previously unexplored area of Peru's southern Amazon Basin. Data on hantaviruses in Peru were sparse and confined to the Loreto Region in the northern Amazon Basin where four cases (three fatal) of human hantavirus infection had been reported in 2011. The team sampled 14 different species of rodents in locations near the construction site for the interoceanic highway and a relatively undisturbed site near the Tambopata National Reserve. A hantavirus sequence was identified in one of two RT-PCR positive rodents (*Neacomys spinosus*) captured near the construction site. Phylogenetic analysis revealed the virus to be an Andes virus clade variant most similar to viruses within the Castelo dos Sonhos (CASV) group, found in Brazil, and Tunari virus (TUNV), found in Bolivia (*Razuri et al. 2014*). This study suggests that the widely distributed *N. spinosus* rodent may be a reservoir for the Andes virus variant, although spillover from an alternate reservoir cannot be excluded.

Arboviruses cause significant illness and death in South America; yet sylvatic cycles and the role of wildlife in the ecology of these viruses is still poorly understood. Outbreaks of disease in wildlife preceding human cases of yellow fever have been recognized in Brazil and Panama suggesting a link between transmission of this virus and wildlife and human population susceptibility. *Mayor et al. (2013)* assessed exposure of hunted wildlife and domestic animals to flaviviruses and alphaviruses in a wildland-rural interface in the northeastern Peruvian Amazon where humans, animals, and vectors had close interaction. Ungulates had the highest seropositivity to flaviviruses and alphaviruses followed by rodents and armadillos and sloths. Ungulates had high titers against St. Louis Encephalitis Virus (SLEV) and Yellow Fever Virus (YFV). Rodents had high titers against Venezuela Equine Encephalitis Virus and SLEV, and armadillos and sloths expressed high

titers against YFV and SLEV. In addition, animals sampled at the relatively disturbed site (i.e. high deforestation and encroachment of land for agricultural use and cattle ranching) had higher seropositivity compared to the more pristine site, suggesting a higher risk of arbovirus infection associated with land-use change (*Mayor et al. 2013*).

LABORATORY METHODOLOGY AND TECHNOLOGY

Because genomic methodology used for viral discovery can be prohibitively expensive and require extensive diagnostic expertise, PREDICT developed a set of relatively simple and inexpensive laboratory protocols for detection of known and novel viruses in high-consequence genera/families. These protocols were used successfully to identify and characterize viruses from PREDICT samples. *Anthony et al. (2013a)* utilized PREDICT viral discovery protocols to investigate the diversity of viruses in bats and provided the first-ever estimate of total diversity of viruses in a mammal species. In addition, PREDICT protocols were used to discover the b-CoV with 96.5% amino acid identity to MERS-CoV in a bat in Mexico (*Anthony et al. 2013b*). These data were used to inform on surveillance of bats in Saudi Arabia, which led to the discovery of a MERS-CoV sequence in a Saudi bat (*Memish et al. 2013*).

PREDICT researchers pushed the leading edge of viral discovery and characterization and developed assays to enhance investigation of zoonotic disease emergence. For example, PREDICT developed an open access bioinformatics computational pipeline (<http://chiulab.ucsf.edu/surpi/>) to rapidly analyze complex metagenomic next-generation sequencing (NGS) data for pathogen identification (*Naccache et al. 2014*). The authors demonstrated use of the pipeline called “sequence-based ultrarapid pathogen identification” (SURPI), which is based on cloud and standalone servers, in the analysis of 1.1 billion sequences originating from clinical human samples. SURPI utilizes SNAP (Zaharia et al. 2011) and RAPSearch (Zhao et al. 2012) sequence aligners to detect known and novel pathogens in less than a 24-hour period. SURPI contributed to the diagnosis for acutely ill patients, illustrating its utility for unbiased NGS-based assays that require rapid reporting (*Naccache et al. 2014*).

In addition, *Grard et al. (2012)* developed and optimized a new and highly sensitive technique to reconstruct the genome of a novel virus in serum from a patient with viral hemorrhagic fever in the Democratic Republic of Congo. This methodology expanded the panel of screening tests when investigating viral hemorrhagic fever cases. In addition, authors created a new serological assay to detect exposure to this novel virus that was used to investigate exposure in persons in close contact with an infected individual (*Grard et al. 2012*). Of interest, *Mokili et al. (2013)* developed methods to detect small circular genomes, such as those of human papillomaviruses, as part of a larger study to develop a standard operating procedure for virus discovery using a metagenomic approach. Furthermore, a number of investigations revealing novel viruses in bats were made possible by advances in high-throughput sequencing (e.g. *Epstein et al. 2010; Quan et al. 2013*).

INFLUENZA VIRUS-SPECIFIC CHARACTERIZATION AND SURVEILLANCE STRATEGIES

PREDICT also published a number of articles that shed light on the diversity, host range, distribution, and seasonality of influenza viruses. *Olson et al. (2013)* estimated the historical prevalence and distribution of influenza A (H7N9) virus in wild bird populations in response to knowledge gaps surrounding the source of infection for the 2013 outbreak of influenza A (H7N9) in the provinces of southeastern China. The apparent prevalence of H7N9 was found to

be historically low in wild birds. The authors estimated that greater than 30,000 wild birds would need to be sampled to detect 1 bird that was H7N9 positive with 95% probability and, therefore, recommended risk-based surveillance as an efficient strategy for monitoring (*Olson et al. 2013*).

Lam et al. (2013) investigated the source populations and the conditions for the genesis of the 2013 H7N9 virus outbreak in China using active surveillance, screening of virus archives, and evolutionary analyses. The authors demonstrated that H7 viruses were likely transmitted from domestic ducks to chickens in China during two separate events (*Lam et al. 2013*). Findings revealed that the H7N9 outbreak lineage originated from reassortment of H7 viruses and enzootic H9N2 viruses (Figure 12). Discovery by the authors of a related H7N7 influenza virus in chickens that has the ability to infect mammals experimentally, suggests that H7 viruses potentially pose a greater threat than previously recognized (*Lam et al. 2013*).

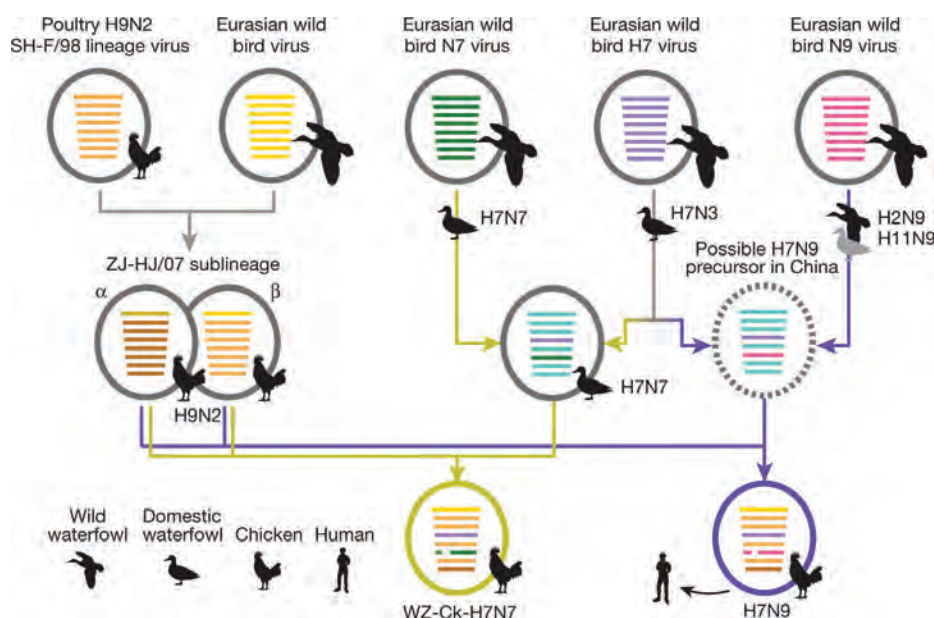


Figure 12. Evolutionary pathways of the H7N9 and H7N7 viruses. Virus particles are represented by ovals with colored horizontal bars for the eight gene segments (from top to bottom: PB2, PB1, polymerase acidic, haemagglutinin, nucleoprotein, neuraminidase, matrix and non-structural). In descendent viruses, the segments are colored according to their corresponding source viruses (top) to illustrate gene ancestry through reassortment events. Source viruses for a reassortment are adjacent to arrow tails, and the arrowheads point to the resulting reassortants. Bars colored cyan indicate gene segments of the ZJ-5 sub-lineage of wild bird viruses. A broken bar in segment 6 (neuraminidase) indicates a stalk region deletion. The virus indicated by a broken oval represents a hypothetical reassortant. From *Lam et al. 2013*.

In addition, *Zhu et al. (2013)* was the first to report H9N2 in wild birds and investigated their role in the ecology of the H9N2 influenza virus. Fifteen H9N2 viruses were isolated from two species of wild ducks (spot billed ducks and mallard ducks) in Poyang Lake of southeast China in 2011. Eleven representative viruses were further characterized by complete sequencing of the eight gene segments. One isolate tested for lethality in laboratory balb/c mice replicated efficiently in mice tissues and led to mortality in 20-40% of infected cohorts, indicating the ability to cause fatal infections in a mammalian species (*Zhu et al. 2013*).

Because live-poultry markets were regarded as the primary sources of human infections with the H7N9 virus during the outbreak, PREDICT assisted with implementation of enhanced surveillance for H7N9 virus at markets in Guangdong, China between April and August 2013. Two H7N9 viral strains were isolated from samples collected at live-poultry markets and from a clinical patient with influenza A infection (*Lu et al. 2014*). Phylogenetic analyses revealed that the H7N9 virus isolated in April/May from environmental and chicken samples was similar to strains isolated in eastern China where most of the human cases were documented. However, the virus isolated in August from the clinical patient in Guangdong was divergent from other reported sequences and was more similar to H9N2 viruses circulating in Guangdong Province, suggesting that reassortment led to the emergence of a novel H7N9 influenza virus (*Lu et al. 2014*). Because reassortment of influenza A viruses may increase their ability to infect people, continued enhanced surveillance and monitoring of H7N9 viruses is important for early detection of novel H7N9 strains that may pose a public health threat.

Olson et al. (2014) summarized knowledge on competent avian hosts of influenza A viruses and patterns of global influenza A subtype distribution (Figure 13). The majority of all known subtypes were found in wild Anseriformes (87%) or Charadriiformes (60%); and geographically the North American Atlantic flyway, Europe, and Asia were hotspots for global richness. To estimate pathogen richness within a study population, as well as to estimate surveillance effort necessary to detect targeted pathogen richness, the authors applied and critically evaluated the use of species accumulation curve methodology. Overall, the research provides guidelines for efficient surveillance of avian influenza A viruses and recommendations to help synthesize pathogen information from host surveillance and ecology to isolation and sequencing.

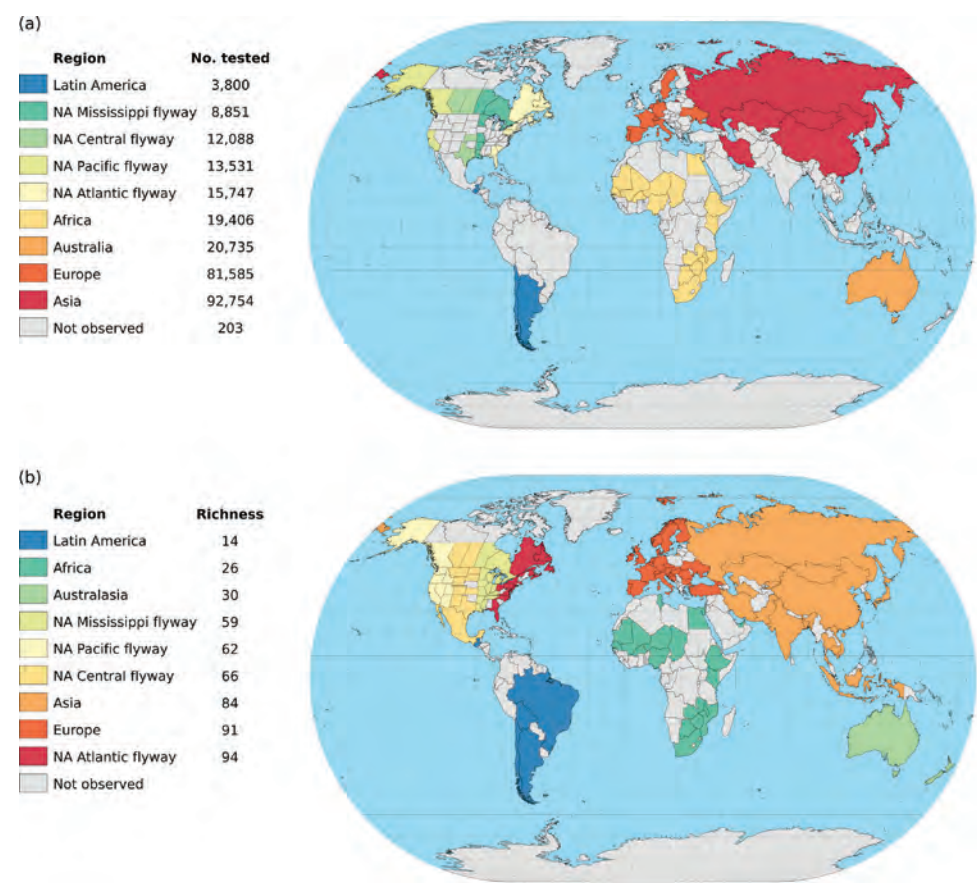


Figure 13. a) Total number of birds tested by region in 50 studies that non-discriminately tested for AIV subtype. If no studies were identified that met the inclusion criteria for a country, state (USA only), or province (Canada only) it is reported as not observed. b) Total number of AIV subtypes detected by region based on records in GenBank and 50 studies. A country, state (USA only), or province (Canada only) is not observed if no studies were identified that met the inclusion criteria and if no subtypes were reported in GenBank. From Olson et al. 2014.

PREDICT also conducted research to investigate drivers of H5N1 persistence. *Hosseini et al. (2013)* used mathematical models of H5N1 dynamics in different-sized poultry farms to understand the virus's ability to persist in different types of poultry operations and to investigate the effects of culling and cleaning as control measures. Results indicated that moderately sized poultry farms can sustain H5N1 for over two years without wild bird involvement. A mixture of intensive/backyard farming within a country could sustain H5N1 or similar influenza virus indefinitely (*Hosseini et al. 2013*). In countries with a need for intensive poultry production, larger scale commercial poultry operations with more intensive H5N1 monitoring and increased biosecurity may be the best strategy for reducing risk of human infection with H5N1 and persistence in poultry (*Hosseini et al. 2013*).

In addition, *Murray and Morse (2011)* assessed whether human H5N1 cases occur seasonally in Indonesia and Egypt in association with changes in temperature, precipitation, and humidity. The incidence of human H5N1 in Egypt, but not Indonesia, was strongly associated with meteorological variables. In addition, incidence of infection was highest in Egypt when precipitation was low, and temperature, along with absolute and relative humidity, were moderate compared to the average daily conditions in Egypt suggesting that human infection may be occurring primarily via droplet transmission from close contact with infected poultry (*Murray and Morse 2011*).

PREDICT also contributed to investigations of influenza viruses spilling over into marine mammal species. *Goldstein et al. (2013)* detected pandemic H1N1 influenza in free-ranging northern elephant seals in 2010 off the central California coast. Virus isolation, whole genome sequencing, and hemagglutination inhibition assay confirmed exposure to pandemic H1N1 influenza virus in the seals. In vitro characterizations showed that replication of the virus was similar to that of reference strains of pandemic H1N1 in canine kidney cells. However, the virus did not replicate well in human epithelial respiratory cells, demonstrating that the virus isolates may be elephant seal adapted. This was the first isolation of H1N1 in a marine mammal. These findings provided evidence for cross-species transmission of influenza viruses among free-ranging wildlife and between wildlife and people and provided evidence that oceanic transmission and movement of pathogens should not be eliminated from consideration of amplification and spread (*Goldstein et al. 2013*). In addition, *Anthony et al. (2012)* documented the emergence of influenza A virus (H3N8) in New England harbor seals which caused an outbreak of pneumonia and contributed to an unusual mortality event. Sequence analysis revealed a H3N8 influenza A virus that is similar to an influenza virus documented in North American waterfowl in 2002 but with mutations consistent with adaptation to mammalian hosts (*Anthony et al. 2012*). At the time of this publication, additional influenza projects were ongoing to help identify productive surveillance targets and evaluate all possible surveillance modalities to improve global efficiencies.

OTHER CONTRIBUTIONS TO ENHANCING OUR UNDERSTANDING OF ZOOONOTIC DISEASES

PREDICT also contributed to research investigating the presence of zoonotic pathogens in hunted wildlife in South America. *Aston et al. (2014)* explored the occurrence of *Toxoplasma gondii* exposure among hunted peccaries, brocket deer, and lowland tapir in the Peruvian Amazon as a model for pathogen sharing. For this study, blood spot samples were obtained from animals hunted in the area surrounding the community of Nueva Esperanza where human cases of ocular toxoplasmosis have been documented. Evidence of exposure was common in the hunted ungulates (17-40%), suggesting a potential source of *T. gondii* infection in this community. Seroprevalence was lower in this study relative to other surveys of wild ungulates in

less remote locations in the Amazon with domestic and feral cats. Similarly, *Gardner et al. (2013)* documented a case of *Echinococcus vogeli* from a lowland paca (*Cunicula paca*) in Bolivia. The paca, the intermediate host in Bolivia, was collected as part of a wildlife health survey carried out on game hunted for consumption by families of Tsimane indigenous hunters. *E. vogeli* is the causal agent of polycystic hydatid disease. While *E. vogeli* has been reported commonly from humans in South America, most infections have been reported from people from countries with relatively well-developed public health reporting infrastructures. Polycystic hydatid disease is now considered the most pathogenic of all cestodiasis, posing a much greater threat to public health than other forms of taeniasis or echinococcosis worldwide. Another study conducted by *Limachi et al. (in press)* on wildlife hunted for food at this same Tsimane community showed that peccaries, the community's most commonly hunted and consumed species, carry several gastrointestinal parasites, including the zoonotic genus *Ascaris* sp. While the *Ascaris* sp. prevalence was low, it may be underestimated as a result of seasonality and age class of the sampled peccaries, given that *Ascaris* sp. are known to be more prevalent during the rainy season and in young animals. Notwithstanding, this study provides the first report for gastrointestinal parasites in peccaries from Bolivia and warrants further investigations into potential peccary-to-human transmission given the close contact existing between Amazon inhabitants and tayassuids.

PREDICT researchers also contributed to a study investigating wild rodents as a potential reservoir for *Leishmania braziliensis*, a zoonotic parasite that is responsible for the prevalent leishmaniasis in the Peruvian Amazon (Madre de Dios; *Shender et al. 2013*). Wild rodents (*Oligoryzomys microtis*, *Hylaeamys perenensis*, and *Proechimys* spp.) were captured along a segment of newly constructed Transoceanic Highway for screening for *Leishmania* parasites to assess risk of infection for people living and working in the disturbed terrain. All of the samples were negative, suggesting that these rodent species are unlikely to serve as primary reservoirs for *L. braziliensis* along the Transoceanic Highway in Madre de Dios (*Shender et al. 2013*).

In addition, *Anthony et al. (2014)* improved our understanding of the geographic distribution of WNV. The researchers documented the expansion of WNV to the British Virgin Islands by identifying its emergence in flamingos. Sequence analysis revealed that the strain is similar to those circulating in the United States since its first emergence in the country in 1999 (*Anthony et al. 2014*).

PREDICT researchers also contributed to a study investigating a recently described close homolog of hepatitis C virus, canine hepacivirus, in order to provide further insights into the origins of hepatitis C virus in people. *Lyons et al. (2012b)* investigated the species distribution and clinical features of non-primate hepaciviruses or homologs using large-scale PCR-based screening on samples from a range of mammalian species. In this study, non-primate hepacivirus was found in three horses and was not associated with pathology (*Lyons et al. 2012b*). In previous studies, canine hepacivirus sequences were detected in samples from horses in the U.S. suggesting a broader host range beyond dogs and leading to new nomenclature to describe the virus (i.e. non-primate hepacivirus). In addition, *Lyons et al. (2014)* screened large numbers of horses for viremia and for past exposure through serological assays for non-primate hepacivirus and equine pegivirus. Approximately 43% of 328 horses were seropositive for non-primate hepacivirus (three viremic), and 66% of horses were seropositive for equine pegivirus (12 viremic). Detection of hepacivirus and pegivirus in a range of mammalian species, including dogs, horses, rodents, and bats (*Quan et al. 2013*; Kapoor et al. 2013; *Lyons et al. 2014*) begins to shed light on the potential for cross-species transmission and improves our understanding of the evolutionary history of these viruses.

CONTRIBUTIONS TO GLOBAL HEALTH POLICY

PREDICT also made contributions to global health policy through participation in featured editorials with the United Nations Convention on Biological Diversity Leadership. For example, *Langlois et al. (2012)* highlighted that health and ecosystems are inextricably linked to all development sectors and outlined policy implications of health on global sustainable development goals. In addition, in collaboration with leadership from the World Health Organization, *Campbell et al. (2012)* outlined policy implications for biodiversity and global health and called for a greater awareness of the need for a more holistic approach by the health and biodiversity professionals.

PREDICT supported the tri-lingual publication of the OIE Scientific and Technical Review focused on the theme of One Health (*Karesh 2014*). In this issue, 64 governmental representatives, organizational heads, and experts on One Health related issues from around the world provided insights and shared experiences to lead readers through the progression of One Health from concepts to perspectives to practices. *Stephen and Karesh (2014)* introduced the publication with a discussion on the question, “Is One Health Delivering Results?” *Mazet et al. (2014)* expounded on the topic of the stakeholders in One Health, and *de La Rocque and Formenty (2014)* provided in-depth guidance on using One Health principles to create an effective framework for preventing and responding to Rift Valley Fever outbreaks.

In addition, PREDICT teams contributed to policy-relevant work as requested to improve global health management and interventions. For example, PREDICT contributed to the first species and globally comprehensive review of Foot and Mouth Disease, including host range and control strategies. The authors highlighted the disease implications for human livelihoods and sustainable economic development efforts (*Weaver et al. 2013*). Additionally, PREDICT conveyed the relevance of conservation policy in animal and human health through consideration of implications of international regulations for rabies control (*Machalaba and Karesh 2012*).

PREDICT, through support of the IUCN Species Survival Commission (SSC) Wildlife Health Specialist Group, also led the development of the OIE-IUCN Guidelines to Wildlife Disease Risk Analysis (*World Organisation for Animal Health (OIE) & International Union for Conservation of Nature (IUCN) 2014*) in coordination with colleagues from RESPOND and the IUCN SSC Conservation Breeding and Invasive Species Specialist Groups. The publication provides an overview of the science-based processes and tools available for wildlife disease risk analysis and their application to a broad range of contemporary issues, including human-wildlife interactions, domestic animal-wildlife interactions, and the impacts of massive ecological change on biodiversity conservation. The guidelines, which serve as a companion volume to the *Manual of Procedures for Wildlife Disease Risk Analysis*, are intended for policy and other decision makers faced with the social, political, and technical complexities involved in wildlife-disease-associated scenarios to enable more proactive consideration of potential risks (*Jakob-Hoff et al. 2014*).

Furthermore, *Pike et al. (in review)* used economic modeling of the cost of pandemic outbreaks to show that 1) current business-as-usual approaches need to be globally coordinated for effective control, and this collaborative approach needs to be implemented within 27 years to maximize benefits – waiting longer makes these programs far less effective; and 2) that globally-coordinated adaptation strategies for pandemic prevention (e.g. Emerging Pandemic Threats program) will be more costly initially, but save money in the long-term over business-as-usual, adaptation programs (e.g. vaccine development; *Pike et al. in review*). Lastly *Perrings et al.*

(2014) highlighted how epidemiological economics (i.e. work at the boundary between ecology, epidemiology, and economics) has the potential to improve predictions regarding the dynamics of epidemics and pandemics and to decrease costs of disease control and prevention compared to traditional measures. Epidemiological economics focuses on the economic causes and epidemiological consequences associated with the numbers and types of contact that individuals make and identifies potential economic incentives to influence contact rates and social mixing (Perrings *et al.* 2014).

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FUTURE DIRECTIONS



Historically, attempts to control deadly viruses, such as SARS and Middle Eastern Respiratory Syndrome (MERS) coronaviruses, influenza, and Ebola, have been almost entirely reactionary. The World Bank estimates that from 1997 to 2009, at least \$80 billion was spent responding to just six outbreaks of deadly zoonotic diseases caused by viruses shared between people and animals (World Bank 2012).

In recognition of the costs of emerging infectious diseases (EID) in both lives lost and dollars spent on treatment and control and the need for a more proactive paradigm, the PREDICT project conducted the most comprehensive zoonotic pathogen and EID surveillance capacity building program in the world to date. As detailed in this report, a risk-based surveillance strategy was used in regional “hotspots” for emerging diseases with the aim of targeting efforts toward early detection and response to potentially high-consequence animal viruses before they become significant public health threats.

In order to further focus surveillance and interventions at the source of pathogen emergence, amplification, and spread in human populations and especially to predict risk from viruses before they emerge, future work should:

- Expand characterization of pathogens of known epidemic and unknown pandemic potential and their dynamics among hosts;
- Document pathogen sharing between wildlife, domestic animals, and people;
- Target surveillance at high-risk pathways for disease emergence, spillover, and spread in order to identify social and ecological drivers of (i.e. factors influencing) pathogen emergence and transmission and determine appropriate targets for intervention; and
- Build on global One Health initiatives for effective collaboration across disciplines and geographic borders.

EXPANDING CHARACTERIZATION OF PATHOGENS OF KNOWN EPIDEMIC AND UNKNOWN PANDEMIC POTENTIAL AND THEIR DYNAMICS AMONG HOSTS

For the PREDICT project, the selection of viral families/genera targeted for testing was necessarily broad. It included families of known zoonotic, epidemic, and pandemic risk, as well as those that, despite their perceived lower pandemic risk, had sufficient abundance and diversity in wildlife to act as useful targets for laboratory capacity building. To increase efficiency for targeting pathogens of highest consequence, future efforts should focus on ranking risk of spillover, amplification, and spread of these newly detected viruses using all available virological, epidemiological, ecological, and host-specific data. The development of modeling and analytical

tools needed to derive both spillover and pandemic risk from viral genetic material and ecological data remains a significant challenge. Developing this analytical toolkit to identify the highest risk viruses is a critical next step and will allow for the prioritization of resources for advanced laboratory characterization of select viruses, as well as better targeted surveillance and viral detection and discovery efforts. A focus on those viruses with previous association with Influenza-like Illness (ILI), Severe Acute Respiratory Illness (SARI), Fever of Unknown Origin (FUO), and hemorrhagic disease in people will also be beneficial.

The study of diseases of unknown origin (DUO) provides an important tool for the identification of new and previously undetected pathogens that have the certain capacity to infect and potentially the ability to spread in human populations. Because current laboratory and health systems are targeted at identification of known pathogens that have caused disease in the past, many diseases go undiagnosed, at best making treatment and control very difficult and, at worst leaving treatment of affected individuals limited to supportive care. Further examination of syndromic surveillance samples is needed to identify novel agents associated with ILI, SARI, FUO, as well as other syndromes, such as hemorrhagic fever and encephalitis. In addition to viruses, the full range of bacterial pathogens should be identified in specimens from DUO patients.

Using this approach will allow advanced characterization to be targeted to new viruses detected from viral families/genera of potential pandemic significance that have a relatively wide range of hosts, are related to known pathogens, are detected in new or unusual host species, or cluster with known pathogens found in other host taxa. Full genome sequencing and virus isolation will allow for accurate taxonomic placement, characterization of virus-host cell receptor interactions, and improved understanding of host diversity and the evolutionary processes that shape viral diversity (e.g. reassortment/recombination). This approach will lead towards more effective intervention, enabling the design of treatments, preventives, and serological assays to investigate human exposure to these agents and facilitate the tracing of novel pathogens back to potential animal reservoirs and amplification hosts in order to identify and mitigate risky behaviors at the source of viral spillover and amplification interfaces.

Communities along the banks of rivers in Kathmandu comprise some of the poorest areas in the city. Both people and livestock living in these settlements have limited access to basic infrastructure (e.g. clean water and treatment of sewage), and these communities fall outside of health care systems.



PHOTO BY PREDICT NEPAL TEAM

DOCUMENTING PATHOGEN SHARING AMONG WILDLIFE, DOMESTIC ANIMALS, AND PEOPLE

Human behaviors and practices are key risk components for pathogen spillover, amplification, and spread. Understanding these behaviors and more comprehensively characterizing the type and frequency of contact among people, livestock, and potential wildlife reservoirs are key next steps to reduce these risks. To investigate interspecies transmission along high-risk pathways for emergence and identify critical control points to prevent pathogen spillover – human, livestock, and wildlife sampling must align in time and space at key high-risk interfaces. Intensive livestock production systems, points

of potential contact along animal value chains, and land conversion for commercialization have been identified as high-risk settings for the emergence of pandemic threats (Figure 1). To improve our understanding of the behavioral mechanisms involved in high-risk pathways, these data should be used to develop analytical models that link specific human behaviors and zoonotic disease risk across sites. It is essential to identify critical control points and risk-mitigation options, field piloting strategies to gauge individual and community willingness and uptake potential in order to determine which interventions might be taken to scale. Critical examination of scenarios that integrate epidemiological models with cost-benefit analyses for different pandemic control strategies can then be used to inform policy makers on the most likely effective interventions.

In addition, it is important to compare novel agents detected in people with diseases of unknown origin to newly discovered animal viruses and bacteria to identify animal hosts and practices heightening risk for spread. Genomic sequencing combined with new approaches that integrate molecular evolution, phylogenetics, and coevolution will be used to help unravel the mysteries that influence pathogen sharing across the animal-human interface. Further, disease outbreaks in people and animals also present unique opportunities for parallel sampling of wildlife, livestock, and people; pathogen discovery; and investigations of spillover events resulting in disease.

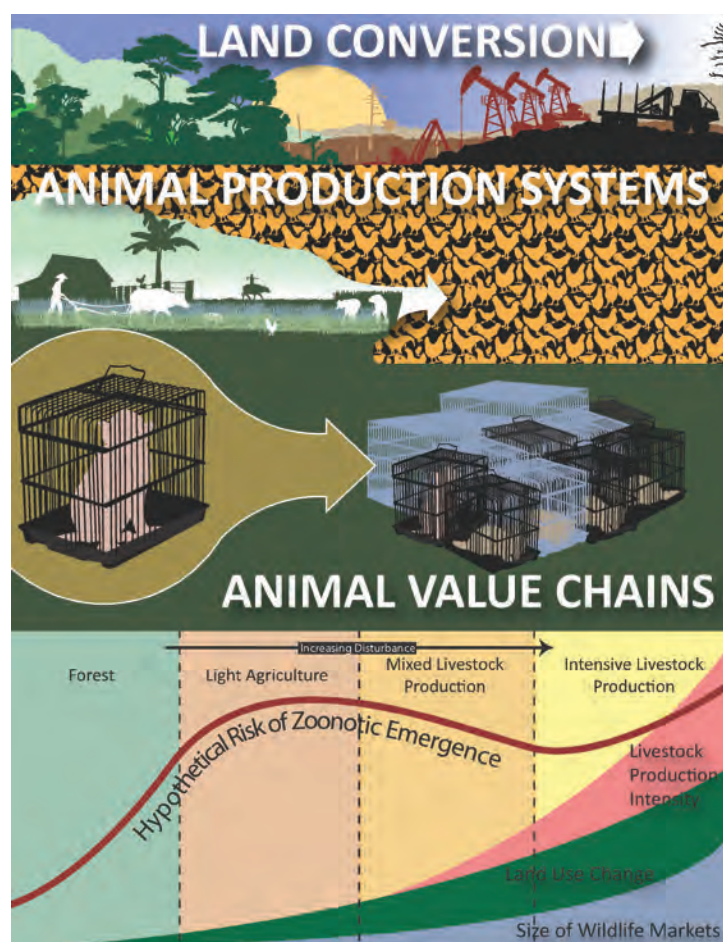


Figure 1. Land conversion for commercialization, animal production systems, and animal value chains are three high-risk pathways for pathogen emergence, spillover, and spread.

IDENTIFYING DRIVERS OF PATHOGEN EMERGENCE AND TRANSMISSION AND DETERMINING POTENTIAL TARGETS FOR INTERVENTION

To increase efficiency for identifying viruses that pose the greatest risk for emergence in people, future efforts should be most focused in areas where environments and systems are changing in ways that are conducive to the spillover of viruses from animals into humans. Rapidly improving, advanced analytical approaches based on quality science, will provide key information on the potential for spillover, amplification, and spread within identified emergence pathways. To better forecast risk of emergence along key pathways, high resolution spatial datasets that include changes in land cover, specific land-use, livestock density, patterns of wildlife trade, and changes in wildlife distribution will need to be analyzed under realistic future scenarios of demographic, environmental, economic, and climate change. Further, an epizone approach (Figure 2), in which the whole geographic, ecological, and sociological space of these disease emergence and transmission pathways is characterized, offers opportunities to improve understanding of pre-spillover conditions that drive viral evolution, transmission of zoonotic pathogens, and circumstances of pathogen amplification and spread.

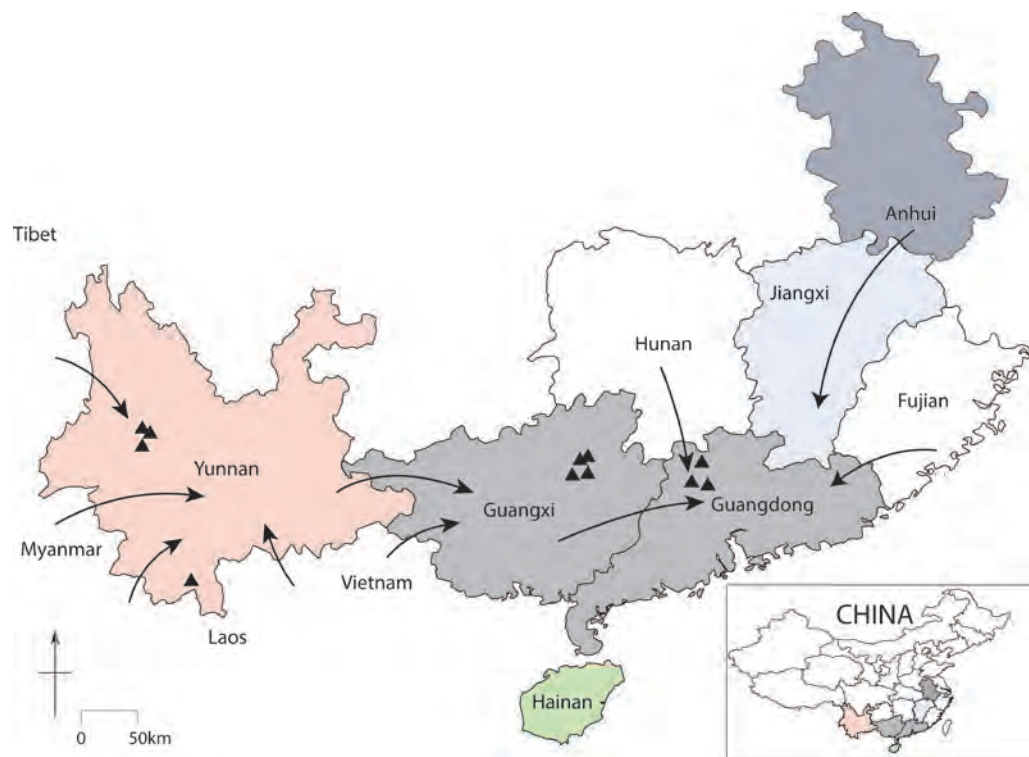


Figure 2. An epizone for SARS Coronavirus includes the wildlife markets of Guangdong where it amplified and spread, but also the whole wildlife trade along Southern China back to Yunnan Province, where PREDICT identified a diverse cluster of bat SARS-like coronaviruses capable of infecting human cells. From Ge et al. 2013.

Identifying key processes influencing the evolution, spillover, amplification, and spread of pathogen threats could likely best be achieved through augmenting and integrating data generated through PREDICT and partner networks with 1) selective longitudinal sampling of wildlife, livestock, and at-risk human populations with a special focus on ILI, SARI, and DUO patients; 2) detection and characterization of pathogens with evidence of infectivity and transmissibility among hosts, including people; 3) simultaneous collection of data on human behaviors, movements, practices, and the ecological conditions governing these aspects of human ecology; and 4) analyses of key viral characteristics in combination with data collected on hosts, ecological drivers, human behaviors and practices, exposure rates, and ecological conditions.

Advanced informational technologies and dynamic analytical frameworks could then be applied to the integrated biological surveillance, behavioral risk characterization, and economic and anthropologic data to identify potential targets for intervention and informed policy development.

BUILDING ON GLOBAL ONE HEALTH INITIATIVES FOR EFFECTIVE COLLABORATION ACROSS DISCIPLINES AND GEOGRAPHIC BORDERS

Through its local and global One Health initiatives, PREDICT and its partners enabled a structure for effective collaboration across disciplines and geographic borders to promote global health through prevention of emerging zoonotic disease threats. This infrastructure can be built upon to include additional regions and stakeholders. In fact, the Emerging Pandemic Threats program has developed two additional new initiatives, PREPAREDNESS and RESPONSE and ONE HEALTH WORKFORCE to build capacity and streamline prevention and control measures. The projects, in collaboration with PREDICT, will build on One Health advancements through:

- Development of national and regional strategies for the sustainability and broadening of One Health initiatives, including systems for disease prevention and early detection;
- Engagement and training of more stakeholders, including representatives from additional ministries, health and industry sectors, and local at-risk communities;
- Facilitation of regional and global collaboration on pandemic prevention and preparedness; and
- Illustrations of proofs of concept, including cost/benefit analyses to demonstrate improved outcomes resulting from a One Health approach.

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In Bangladesh, dense human and livestock populations residing in fragmented landscapes that also serve as wildlife habitat have led to greater interactions among people, livestock, and wildlife.

PHOTO BY PAULINA ZIELINSKA, UCD (LEFT) AND BY JON EPSTEIN, EHA (RIGHT)

BANGLADESH

Bangladesh is among the most densely populated countries on Earth, and yet, it is still rich in floral and faunal biodiversity. However, several studies indicate that 4 to 5% of faunal species (Mukul 2007) and about 10% of floral diversity (Khan et al. 2001; Dey 2006) in Bangladesh have become extinct in the last century (Mukul et al. 2008). Due to immense population pressures; over-exploitation of natural resources; habitat loss and degradation; pollution; and indiscriminate killing, hunting, and poaching of wild animals, Bangladesh's environmental and ecological balance is under severe stress, leaving it even more vulnerable to the impacts of global climate change. Bangladesh is particularly susceptible to climate change as a result of increased frequency of natural disasters such as floods and cyclones. Losses in arable lands due to flooding pose a major threat to the food security and livelihoods of rural communities.

Bangladesh provides a unique setting for human, domestic animal, and wildlife interaction due to the co-occurrence of species-rich landscapes and high density human communities that depend heavily on intensive livestock production and also on domestic animal labor to drive agriculture. The high density of people and livestock residing in fragmented landscapes that also serve as wildlife habitat, coupled with increased sharing of scarce water and food resources, heightens the risk of zoonotic disease threats and may facilitate transmission of viruses of pandemic potential between animals and humans.

Dhaka is a city of more than 12 million people connected to other major cities around the world via a busy international airport. Many of those who live and work in Dhaka travel to their home villages during festivals and religious holidays, creating ongoing linkages between rural and urban environments and the potential for pathogen spread into a globally connected metropolis.

FOR A SUMMARY OF THIS SECTION GO TO WWW.BANGLADESH.PREDICT.GLOBAL

Bangladesh is a hotspot for emerging and re-emerging infectious diseases. Here, human and animal health are intrinsically linked, and livelihoods are highly dependent upon natural resources. Further, there is little capacity for detecting pathogens that emerge in animals prior to the occurrence of an outbreak in people. The country has recently experienced outbreaks of high and low pathogenic avian influenza (Zaman et al. 2009), H5N1 (Ahmed et al. 2010) and H1N1 pandemic influenza (Ahasan 2009), Nipah encephalitis (Luby et al. 2009), and anthrax (Chakraborty et al. 2012). As Bangladesh has one of the highest densities of human and poultry populations (World Bank 2013), there is a high risk of rapid spread in the event of an emerging virus of pandemic potential. In addition, Nipah virus continues to be a threat in at-risk rural communities in Bangladesh, with annual outbreaks with an average mortality rate of 70%. It is also a global threat, as it can be transmitted from person to person (Luby et al. 2013). These outbreaks illustrate Bangladesh's vulnerability to large-scale epidemics of pandemic potential. It is extremely likely that the high human and animal population densities and the frequent interactions between animals and humans will continue to result in the emergence of other novel, potentially pandemic pathogens in the future.

The speed with which pathogens can emerge and spread presents serious public health, economic, and development concerns and demonstrates the need for the development of comprehensive disease detection and response capacities, particularly in hotspots like Bangladesh. Effective prevention and control of emerging diseases requires an integrated One Health approach. In Bangladesh, the PREDICT project is using a One Health approach to enhance disease surveillance systems, strengthen capacity to characterize zoonotic disease risks, improve understanding of pathogen spillover and spread, and assist the country in preparedness for response to zoonotic disease outbreaks.

PARTNERS

PREDICT partners in Bangladesh included EcoHealth Alliance (EHA), International Centre for Diarrheal Disease Research, Bangladesh (icddr,b), USAID, and Center for Infection and Immunity at Columbia University (CII).

Additional partners included:

- Bangladesh Forest Department
- Institute of Epidemiology, Disease Control and Research (IEDCR)
- Bangladesh Department of Livestock Services (DLS)
- Chittagong Veterinary and Animal Sciences University (CVASU)
- CSIRO Australian Animal Health Laboratory (AAHL)

Children playing with a bat.



PHOTO BY PAULINA ZIELINSKA, UCD

PREDICT is identifying new viruses that exist in common wildlife, such as macaques, at human-animal interfaces with high risk for disease transmission.

MAJOR ACHIEVEMENTS

- Provided the first estimate of the viral diversity in a mammal species and the number of samples needed to maximize viral discovery while minimizing cost (see Success Stories for more information).
- Presented at One Health meetings and conferences across the country and conducted training workshops with FAO on One Health, thus reaching a much broader audience.
- Participated in important One Health Bangladesh workshops that brought the Ministry of Health, the Department of Livestock Services, and the Forest Department together for the first time. The Director General of each ministry signed a document indicating that they will follow One Health principles within their respective ministries.



PHOTO BY ARIFUL ISLAM, EHA

- Trained 29 biologists, students, and veterinarians, including the first veterinarian hired by the Bangladesh Forest Department, in collaboration with PREDICT's in-country partner, International Centre for Diarrheal Disease Research, Bangladesh
- Collected samples from 5,796 animals during the project. A total of 2,435 bat samples were tested by PCR for 11 viral families, 840 rodent and shrew samples were analyzed by PCR for 10 viral families, and 466 nonhuman primate samples were analyzed by PCR for 34 viral families and genera.
- Partnered with icddr,b, which has a molecular biology laboratory with equipment and technical expertise to screen wildlife samples. Introduced the PREDICT PCR protocols, primers, and positive controls to augment lab surveillance capabilities.
- Supported the government and icddr,b's ongoing response efforts during annual outbreaks of Nipah virus.
- Supported IEDCR laboratories by providing PREDICT PCR protocols to be used for human surveillance activities.

SUCCESS STORIES

PREDICT-Bangladesh provided the first ever estimate of total diversity of known and novel viruses in a mammalian species (Anthony et al. 2013). The team used a novel strategy that integrated concepts from viral discovery, phylogeny, and ecological analyses to estimate the approximate viral diversity in the Indian flying fox (*Pteropus giganteus*), a native frugivorous bat.

Training a veterinarian from the Bangladesh Forest Department in sampling wild birds for avian influenza.



PHOTO BY ECOHEALTH ALLIANCE

The PREDICT team repeatedly sampled these bats and utilized archived samples to conduct deep sequencing for viral discovery on over 1,800 individual bats. This approach resulted in the discovery of 55 viruses belonging to nine viral families known to include zoonoses. Using ecological analyses developed to predict biodiversity of species in ecosystems, the PREDICT team estimated that the total diversity in these viral families in this bat species is 58, suggesting that the surveillance strategy was effective in identifying the majority of novel viruses that may be shed by a given species. From this effort, the PREDICT team estimated that there are approximately 320,000 total viruses in mammals globally, and that discovery of 80% of the unknown global diversity of viruses in mammals would cost approximately USD \$1.4 billion – a fraction of the estimated \$30 billion in damages inflicted by just one outbreak – the SARS virus pandemic (Anthony et al. 2013).

CAPACITY BUILDING

PREDICT-Bangladesh used a collaborative One Health approach to build capacity for detection and characterization of novel wildlife viruses that potentially pose a significant public health threat.

Training

PREDICT trained over 29 individuals in topics including zoonotic diseases, sampling methodology, human and animal safety during wildlife capture, personal protective equipment (PPE), and laboratory methodology and safety. Trainees included the Bangladesh Forest Department's expanding veterinary staff in collaboration with icddr,b. The veterinarians were provided training in basic epidemiology, disease surveillance in wildlife, and outbreak investigation. The PREDICT Bangladesh team also worked with the PREDICT Nepal team to assist with bat sampling and train local team members to safely collect and handle bat samples.

We also conducted trainings at local government and FAO-sponsored One Health meetings, and through these meetings, PREDICT shared techniques and technologies and strengthened collaborations among the Bangladesh Forest Department, Department of Livestock Services, IEDCR, and Chittagong Veterinary and Animal Sciences University.

Training One Health participants. The Bangladesh team held several workshops and trainings in association with FAO and One Health Bangladesh on proper personal protective equipment use and sampling techniques.

Laboratory Capacity

To enhance laboratory capacity in Bangladesh, PREDICT PCR protocols (which target viral families and allow for the detection of unknown viruses) and positive controls were used in wildlife sample testing at the icddr,b laboratory and will be provided to IEDCR where they will be used for human disease surveillance.

Building a One Health Network

Supporting a One Health framework in Bangladesh is an important focus for PREDICT personnel who are members of One Health, Bangladesh (OHB) and the One Health Alliance of South Asia (OHASA), a regional One Health network. The PREDICT team participated regularly in OHB meetings, workshops, and One Health conferences. The team was also



PHOTO BY ECOHEALTH ALLIANCE

involved in developing a strategic framework for a One Health approach to infectious diseases in Bangladesh with the Ministry of Health, the Department of Livestock Services, the Forest Department, USAID, and iccdr,b. These activities reflect the commitment that these government agencies made to incorporate One Health into their mandates.

SURVEILLANCE

The surveillance strategy in Bangladesh began with targeted sampling of mammals and wild birds that had a high degree of contact with people in rural and urban settings but quickly focused down on three key taxonomic groups that were highly associated with people and that were most likely to harbor zoonotic viruses: bats, rodents, and nonhuman primates.

Several key high-risk disease transmission interfaces were targeted; however, due to the population density of Bangladesh, the team focused on the peri-domestic interface by sampling animals in and around dwellings (77% of sampled animals). Other high-risk interfaces were targeted by sampling animals that were raiding markets; animals that have contact with researchers, park personnel and tourists; and animals that have little to no contact with people but live in locations where indigenous people hunt (Figure1 and Table 1).

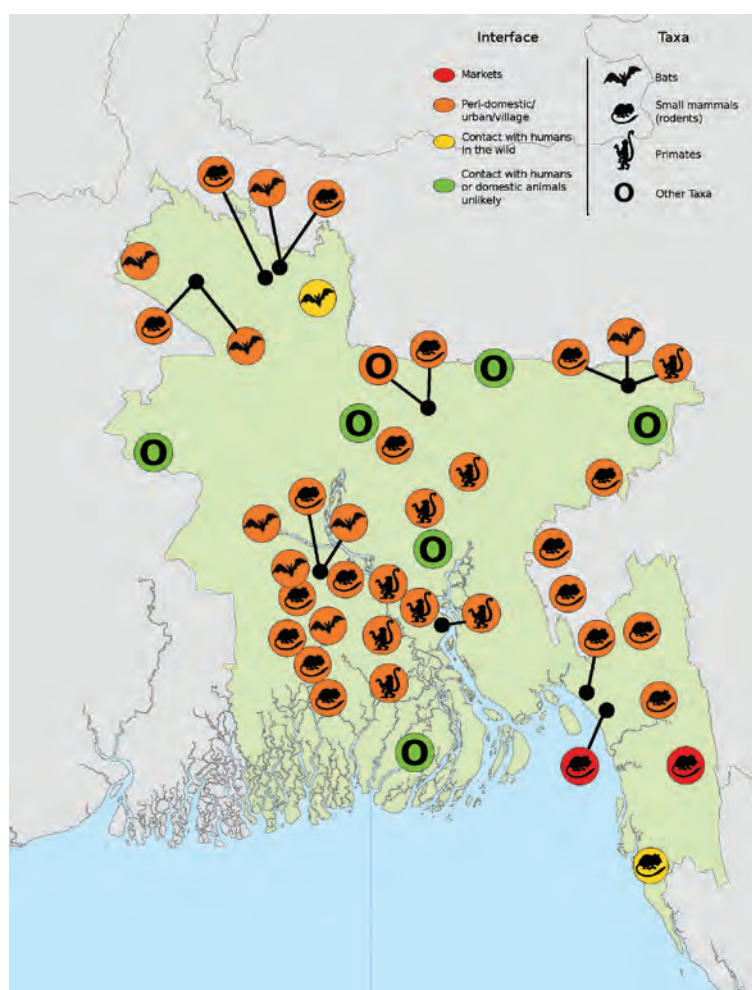


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

A total of 5,796 animals (4,110 bats, 732 rodents and shrews, 543 nonhuman primates, 332 birds, 50 ungulates, eight carnivores, and 21 animals from other taxa; Figure 2) were sampled. A large number of shrews were sampled along with the rodents because, in Bangladesh, shrews are more common in human dwellings than rodents.

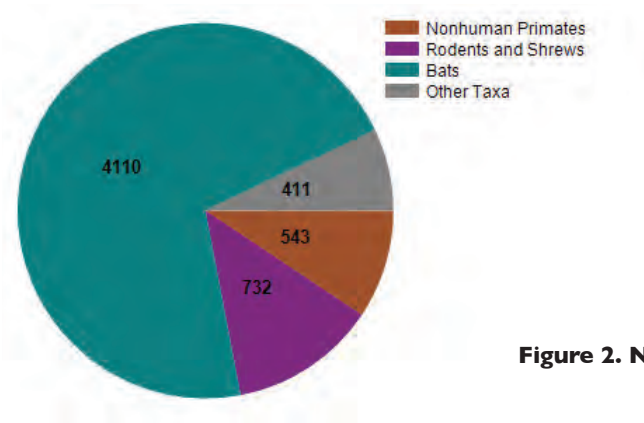


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	0	0	40	0
Ecotourism and recreational activities	4	19	0	0
In or near human dwellings	539	371	3905	28
Wildlife raiding markets	0	84	51	0
Pristine habitat	0	70	114	383
Other high-risk interfaces	0	188	0	0
Total:	543	732	4110	411

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

Bangladesh has experienced repeated outbreaks of zoonotic diseases including Nipah virus, avian influenza, and cutaneous anthrax. In response, the Bangladesh government has developed a sophisticated One Health approach to zoonotic disease outbreak response. PREDICT staff supported the Government of Bangladesh and local implementing partners at the Ministry of Health (IEDCR) and icddr,b by conducting wildlife sampling and training of Forestry Office staff in the safe collection of wildlife samples during Nipah virus outbreak investigations.

VIRUS DISCOVERY AND CHARACTERIZATION

Using data generated from surveillance activities in Bangladesh, PREDICT provided proof-of-concept for a strategy to estimate viral richness within one mammalian species. PREDICT viral discovery protocols were applied to specimens from repeatedly sampled *Pteropus giganteus* fruit bats to saturate the discovery of new viruses (Anthony et al. 2013). This species was selected

because of its role as the reservoir for Nipah virus, which is transmitted through contamination of date palm sap with saliva or urine when bats feed on the sap overnight. Date palm sap is an important interface for bat-to-human spillover of Nipah virus, and may also represent a potential route of transmission for some of the newly discovered viruses as well. Among samples tested from 1,800 bats, 50 novel and 5 known viruses (based on clade analysis) from nine viral families (coronaviruses, paramyxoviruses, astroviruses, influenza A viruses, adenoviruses, polyomaviruses, bocaviruses, and herpesviruses) were discovered.

Analyses commonly used for estimating biodiversity in ecological studies revealed that there are a minimum of 320,000 mammalian viruses awaiting discovery within these nine families assuming all species harbor a similar number of viruses with minimal turnover between host species. The team also predicted that 80% of the viruses in a species could be estimated through testing of only 500 samples from individual animals (Figure 3).

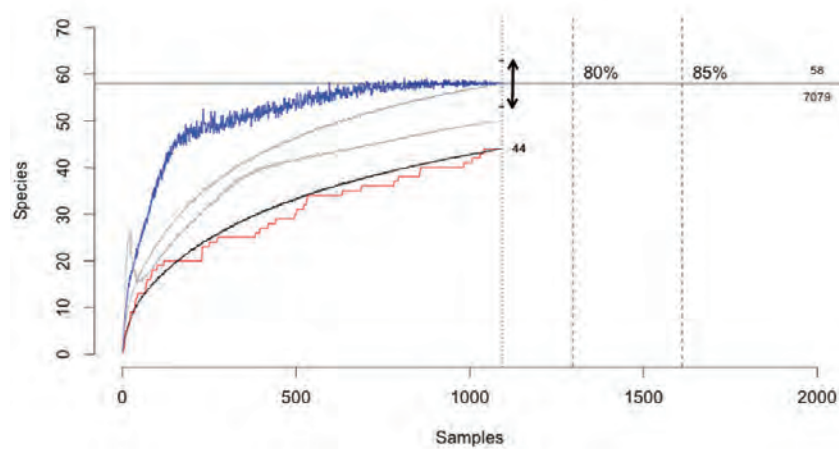


Figure 3. Viral discovery curve. A subset of 1,092 samples were tested for all nine viral families and 44 viruses (out of a total of 55) are represented. The 11 viruses not considered were PgHV-2, -5, -6, and -9; PgAdV-1 and -10, PgAstV-4, -5, -6, and -8, and PgBoV-1. Black line, the rarefaction curve; redline, collector curve showing accumulation of novel viruses over samples tested; blue line, Chao2 estimator at every sample point, with arrow indicating 95% confidence intervals; gray lines, ICE and Jackknife estimators at every sample point; dashed vertical lines, required sampling effort to discover an arbitrary proportion of the total diversity (including 100%); horizontal line, total estimated diversity, 58 viruses, and effort required to discover 100% of the estimated diversity, 7,079 samples. From Anthony et al. (2013).

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In Bolivia, human migration from urban to forested areas is increasing, often following infrastructure development and extractive industries, thereby bringing urban residents into close contact with wildlife.

PHOTOS BY JONNA MAZET, UCD

BOLIVIA

The Amazon is a high diversity 7.8 million km² habitat distributed throughout eight countries and is home to 33 million people including 385 indigenous territories (RAISG 2012). At the global level, the Amazon is seen as the most important source of fresh water and biodiversity in the world and a key player in the planet's climate regulatory system, acting as a natural sink for greenhouse gases. Over the last 50 years, the Amazon region has been the focus of many strategies to improve local, national, and international economies. Currently, the Amazon is considered an important foundation for energy sovereignty and an important source of income originating from the harvest and trade of raw materials (RAISG 2012). However, increased dependence on the Amazon for its natural resources has resulted in significant disturbance to natural areas associated with the expansion and intensification of agriculture, long-distance live animal transport, live animal markets, and an increase in bushmeat consumption. The resulting anthropogenic disturbance is threatening the integrity of natural areas and increasing human contact with wildlife and their pathogens, favoring the emergence and re-emergence of zoonotic diseases.

The Bolivian portion of the Amazon represents 6.2% of the Amazon region and occupies half of the Bolivian territory. It is estimated that between 2000 and 2010, almost 765,000 hectares (1.6% of the Bolivian Amazon) were deforested for agriculture (RAISG 2012). Additionally, 15% of the Bolivian Amazon is under oil exploitation (23% located within indigenous territories), while 0.8% is being subjected to mining. Human migration from urban to forested areas is increasing, often following infrastructure development such as highway and dam construction, gas and oil operations, and timber extraction and mining, thereby bringing urban residents into close contact with wildlife.

FOR A SUMMARY OF THIS SECTION GO TO WWW.BOLIVIA.PREDICT.GLOBAL

The increasing disturbance of the Bolivian Amazon landscape is driving the emergence and re-emergence of zoonotic diseases. In the 1980s, 53% of 350 oil exploration workers were infected with cutaneous leishmaniasis during their incursions into the pristine forest in the Alto Beni region (Desjeux et al. 1987). In 2002, two cases of hantavirus pulmonary syndrome (HPS) were confirmed in two men living and working in an area of extensive anthropogenic disturbance where natural forest was being cleared to cultivate sugarcane (Carroll et al. 2005). More recently in 2013, three cases of HPS (two fatal) were diagnosed in workers from a new sugar mill in the Amazonian municipality of San Buenaventura; these were the first cases of hantavirus infections reported in La Paz (SEDES La Paz 2013).

Due to the lack of efficient systems to report diseases in animal and human populations, zoonotic events are usually underreported. This under-recognition of disease may cause serious social and economic burden in the region, including disruption of family dynamics and high costs to the regional and national health systems. Unfortunately, most of these negative impacts affect already impoverished populations, such as indigenous people or migrant workers from the high-Andes regions who are likely to be more susceptible to tropical diseases.

Because of the establishment of new human-wildlife interfaces in the Bolivian Amazon, the almost inexistent reach of the veterinary service, and the deficient human health care system in this area, a One Health approach to evaluating pathogen dynamics and the inherent risks to surrounding human populations is paramount to detecting, preventing, and controlling emerging zoonotic diseases. In collaboration with several local partners, PREDICT-Bolivia enhanced capacity for wildlife disease surveillance and a One Health approach to disease outbreak investigation.

PARTNERS

The PREDICT-Bolivia team was a collaboration between local and international organizations including: the Wildlife Conservation Society (WCS), EcoHealth Alliance (EHA), USAID, the Centro de Estudios en Biología Teórica y Aplicada, Programa para la Conservación de los Murciélagos de Bolivia (BIOTA - PCMB), and the Institute of Molecular Biology and Biotechnology of Universidad Mayor de San Andrés (IBMB).

Other local partners included:

- National Veterinary Service (SENASAG)
- General Directorate of Biodiversity and Protected Areas (DGBAP)
- Ministry of Public Health (MoH, Zoonoses, and Hantavirus/Hemorrhagic Fever Programs)
- Beni Regional Department of Health (SEDES Beni)
- Pan-American Health Organization (PAHO Bolivia)
- Instituto Nacional de Laboratorios en Salud (INLASA)
- Instituto de Investigación Técnico Científica de la Universidad Policial (IITCUP)
- Institute of Ecology from the Universidad Mayor de San Andrés, La Paz (IE - UMSA)
- Veterinary School of Universidad Católica Boliviana, Unidad Académica Campesina de Carmen Pampa (UCA-UAC CP)



PHOTO BY MELINDA ROSTAL EHA

Participants of the Bolivian Bat Conservation Program and EcoHealth Alliance training workshop on bat capture and sampling methods for PREDICT collaborating field veterinarians and biologists.

- Biology School of Gabriel René Moreno University in Santa Cruz
- Museo de Historia Natural Noel Kempf Mercado
- La Senda Verde (LSV) wildlife rescue center
- Comunidad Inti Wara Yassi (CIWY) wildlife rescue centers
- Consejo Indígena del Pueblo Tacana (CIPTA)
- Consejo Regional T'simane Mosen (CRTM)
- Tierra Comunitaria de Origen Quechua-Tacana San José de Uchupiamonas
- Parque Nacional Madidi
- Reserva para la Biósfera Pilon Lajas
- Zoológico Municipal Vesty Pakos
- Red Boliviana Contra el Tráfico de Animales Silvestre (REBOCTAS)

MAJOR ACHIEVEMENTS

- Significantly improved capacity for disease outbreak response through a coordinated effort to identify and assist with the response to a yellow fever outbreak in free-ranging red howler monkeys in 2012 (see Success Stories for more information).
- Built laboratory capacity at the Institute of Molecular Biology (University of San Andres, IBMB lab) for viral genus/family level PCR on wildlife samples.

- Transferred diagnostic technology to the two most important national public health labs, Centro Nacional de Enfermedades Tropicales - National Center for Tropical Diseases (CENETROP) and Instituto Nacional de Laboratorios de Salud - National Institute for Public Health Laboratories (INLASA).
- Greatly increased knowledge and awareness of wildlife-origin zoonotic pathogens among public health agencies, organizations, and people at risk of exposure.
- Characterized the wildlife trade in Bolivia, in collaboration with the Bolivian Network against the Illegal Wildlife Trade (REBOCTAS), to provide enforcement agencies with information critically needed to identify priorities and strategies for trade regulation.
- Partnered with indigenous hunters and communities for wildlife disease surveillance of subsistence hunted animals, greatly expanding the reach of surveillance efforts and directly engaging a primary stakeholder group.
- Collaborated on the development of a National Zoonoses Surveillance Plan to formalize inter-agency cooperation for zoonotic disease surveillance, outbreak response, and risk communication.

SUCCESS STORIES

One Health Approach to Yellow Fever Outbreak

PREDICT significantly improved capacity for disease outbreak response in Bolivia through a coordinated effort to identify and assist with the response to a yellow fever outbreak in free-ranging red howler monkeys (*Alouatta sara*) in 2012. After six howler monkey carcasses were found near the Ambue Ari Wildlife Sanctuary Park by sanctuary staff, post-mortem examinations were performed at the Municipal Zoo in La Paz. Liver samples were tested using PCR at the Institute of Molecular Biology at the University of San Andres and were found to be positive for a flavivirus.

Within eight days of the onset of the outbreak, the Head of Epidemiology at the Ministry of Public Health, the Coordinator of the Yellow Fever Program at the Pan-American Health Organization (PAHO) in Bolivia, and the General Directorate of Biodiversity were notified of the results, which prompted implementation of preventive measures in the surrounding area, including public education, vaccination campaigns, and mosquito control. Further assessment, including sequencing and phylogenetic analysis of the virus sequences revealed that the outbreak was caused by two yellow fever viral strains, both of which were related to previous human cases of yellow fever in Trinidad and Tobago and Brazil.

No human cases of yellow fever occurred during the outbreak (Alandia et al. 2013), which may be due in part to the rapid response by the Bolivian government. Neotropical primates are highly vulnerable to yellow fever, and outbreaks in howler monkeys can be used as an early indication of risk for people. The yellow fever event helped to strengthen alliances with the Bolivian government, USAID, PAHO Bolivia, and other partners for outbreak response and illustrates the benefit of a coordinated One Health approach to outbreak response.

Post-mortem examination of a howler monkey affected by yellow fever conducted at the Municipal Zoo “Vesty Pakos”, La Paz.



PHOTO BY ERIKA ALANDIA, WCS

CAPACITY BUILDING

Development of a National Zoonoses Surveillance Plan

In the three years that PREDICT was active in Bolivia, capacity for detection of and response to emerging zoonotic pathogens of wildlife-origin was significantly enhanced. At the national level, PREDICT helped improve Bolivia's capacity for disease surveillance and outbreak response by working collaboratively with government agencies through technical advice, field investigations, and training. Given the significant increase in the number of cases of severe zoonotic diseases reported across Bolivia, PREDICT encouraged an inter-agency initiative to develop a National Zoonoses Surveillance Plan. The initiative included the Ministry of Public Health (MoH), the Veterinary Service (SENASAG), the General Directorate of Biodiversity (DBG), and the local office of the Pan-American Health Organization (PAHO Bolivia). Workshops were held in December 2011 and March 2012 to identify gaps and define institutional responsibilities and capacities for conducting zoonoses surveillance. The MoH coordinated the drafting of a new regulatory framework, to formalize inter-agency cooperation for zoonotic disease surveillance, outbreak response, and risk communication. This framework is expected to provide sustainability for policy directions, despite frequent turnover in institutional leadership and officers, and thus to help ensure sustainability of the National Zoonoses Surveillance Plan.

Intersectoral Cooperation

In order to develop a sustainable surveillance program, PREDICT facilitated inter-ministerial forums to design national strategies for wildlife disease surveillance. Key partnerships were formalized with nine institutions, including ministries (General Directorate of Biodiversity



PREDICT country coordinator, Erika Alandia, explains PREDICT methodology for wildlife sampling at the Municipal Zoo “Vesty Pakos”, La Paz, during a training workshop on wildlife surveillance.

PHOTO BY WILDLIFE CONSERVATION SOCIETY

and Protected Areas (DGBAP)), laboratories (IBMB, Instituto Nacional de Laboratorios en Salud (INLASA) and Instituto de Investigación Técnico Científica de la Universidad Policial - IITCUP), universities (Major University of San Andrés, La Paz (UMSA), Universidad Católica Boliviana, Unidad Académica Campesina de Carmen Pampa (UCA-UAC CP)), NGOs (WCS and BIOTA-PCMB), and civil organizations (La Senda Verde (LSV) wildlife rescue center).

Training

PREDICT provided training on wildlife surveillance methods to a wide range of participants in order to build capacity for sustainable development of wildlife disease surveillance systems in Bolivia. A total of 178 people were trained, including field staff; veterinarians; biologists; laboratory technicians; government personnel from public health, veterinary services, and other sectors; wildlife rescue centers staff; and indigenous community residents. Training covered a variety of topics, such as biosafety, animal capture, standardized data and sample collection, cold chain maintenance, sample packing and shipment, and safe laboratory operations. Biosafety was strongly emphasized to prevent occupational risks, such as the potential exposure of field and laboratory personnel to animal pathogens during sampling, post-mortem examination, and specimen handling. Questionnaires were completed at the end of the training sessions to ensure effective knowledge transfer.

Diagnostic Laboratory Capacity

Broadly reactive consensus PCR assays were implemented at PREDICT's partner laboratory (IBMB) for detection of 12 different viral genera/families of public health importance in bats, rodents, and nonhuman primates (alphaviruses, arenaviruses, bunyaviruses, coronaviruses, filoviruses, flaviviruses, hantaviruses, henipaviruses, orthomyxoviruses (influenza), paramyxoviruses, poxviruses, and rhabdoviruses).

SURVEILLANCE

Wildlife disease surveillance was conducted in bats, nonhuman primates, and rodents at human-wildlife interfaces where pathogen transmission is most likely to occur in Bolivia (Figure 1 and Table 1): subsistence hunting by indigenous communities, wildlife trade, captive wildlife settings (e.g. sanctuaries, rehabilitation/rescue centers), peri-domestic settings (i.e. in and around human dwellings or fields), extractive industries (e.g. logging), and animal agriculture (i.e. livestock). In addition, PREDICT conducted wildlife disease surveillance during disease outbreaks in people. Wild animals were also sampled in remote protected areas without human disturbance for baseline comparison. Samples were collected from 1,608 animals, including 289 rodents, 751 bats, 142 nonhuman primates, 144 ungulates, 21 carnivores, and 261 animals from other taxa (Figure 2).

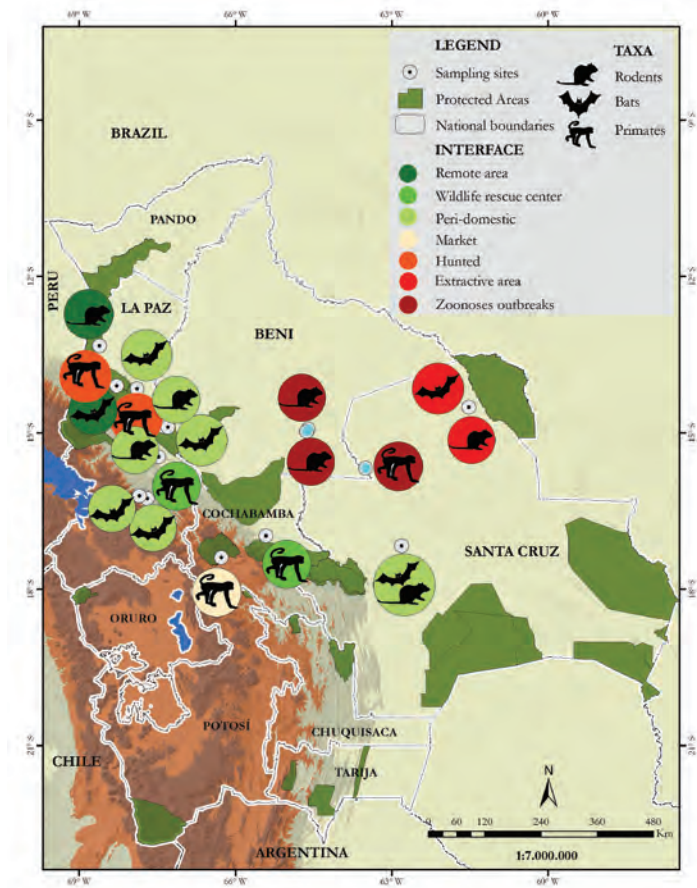


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

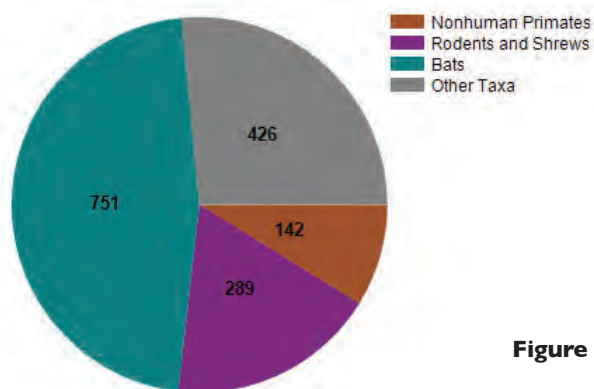


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	0	6	0	0
Extractive industries	0	13	25	1
In or near human dwellings	0	211	328	0
Hunted wildlife	72	44	60	226
Wildlife preying on livestock or their food	0	11	17	1
Pristine habitat	0	0	185	0
Wildlife trade	2	0	0	53
Markets	2	0	0	71
Wildlife being studied	0	4	0	1
Protected areas	51	0	0	72
Zoos and sanctuaries	15	0	0	1
Other high-risk interfaces	0	0	136	0
Total:	142	289	751	426

Previous work conducted by WCS laid the foundation for surveillance of hunted wildlife in indigenous territories and free-ranging wildlife in remote areas of the Amazon rainforest. The WCS five-year community-based monitoring program and reporting of livestock diseases by indigenous communities in the northern Bolivian Amazon set the stage for PREDICT staff to carry out collaborative wildlife sampling activities in this area. Field staff accompanied Tacana, Uchupiamonas, and T'simane subsistence hunters to collect wildlife samples, and two local indigenous technicians were trained to collect specimens from hunted wildlife when PREDICT staff were not present. Because maintaining a cold chain was not feasible in these remote areas, filter paper was employed to collect blood samples (e.g. dried blood spot cards).

Bat samples were collected from Madidi National Park as part of a larger project to identify bat species along the Tuichi River, where two new bat species were reported for that protected area. In addition, collaborations with the Noel Kempf Natural History Museum and the Biology School of Gabriel René Moreno University in Santa Cruz facilitated surveillance of bats and rodents in and around human dwellings and in timber extraction areas. Bats living in and around human dwellings from the Yungas of La Paz were also sampled in collaboration with the Veterinary School of the Unidad Académica Campesina of Carmen Pampa from the Catholic University of La Paz. These sampling events provided opportunities to promote multidisciplinary and inter-institutional One Health education among biology and veterinary students. Other key partners, including indigenous animal health promoters and staff from parks, zoos, and rescue centers, were instrumental in efforts to establish a national wildlife disease surveillance network and committed to reporting animal disease events at wildlife-human interfaces.

Surveillance was also conducted in partnership with government agencies in Bolivia (i.e. Ministry of Health and Servicio Departamental de Salud del Beni – SEDES Beni), including a rodent sampling campaign during a disease control program and an outbreak investigation of arenavirus hemorrhagic fever in people. Furthermore, a partnership with the General Directorate of

Biodiversity and Protected Areas (DGBAP) allowed for wildlife disease surveillance in the wildlife trade through sampling of confiscated animals.

Local Media Surveillance

Bolivia participated in the Local Media Surveillance (LMS) study (see Information Management section and Schwind et al. (2014) for more information). In Bolivia, the LMS pilot study was conducted during a 16-week period to survey health-related news across the country. PREDICT-Bolivia scanned print newspapers (e.g. “La Palabra del Beni”, “Página Siete”) on a weekly basis and reported 12 disease events and 12 risk events in-country, which generated 24 local media surveillance alerts on HealthMap. The most common diseases included arenavirus hemorrhagic fever, hantavirus infections, dengue, canine and bat-borne rabies, leptospirosis, and Chagas disease.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

The PREDICT team worked collaboratively with government agencies to improve the infrastructure and personnel capacity for responding to and investigating outbreaks in the Amazon. PREDICT was invited to join the National Veterinary Service and the Ministry of Health epidemiology teams to respond to three zoonotic disease outbreaks associated with wildlife (yellow fever, rabies, and arenavirus hemorrhagic fever). In addition to using a One Health approach to the yellow fever outbreak response in howler monkeys, PREDICT also engaged in government response efforts to a case of hemorrhagic fever in a member of the community of Elvira in the northern Bolivian Amazon in 2011, as well as an outbreak of rabies in llamas (*Lama glama*) in Potosi in 2011.

Response efforts were focused on active surveillance of wild animal reservoirs and training of agency, university, and NGO personnel on wildlife sampling and disease reporting to the animal and public health authorities.

The human case of hemorrhagic fever in Elvira was located outside of the hemorrhagic fever endemic area in Bolivia. Recent land-use changes in this region, such as introduction of rice crops by newly established settlers, may be facilitating growth of rodent populations, and therefore increasing the risk of spillover of rodent-borne zoonoses such as arenavirus hemorrhagic fevers. With approval from the National Vice-Minister of Public Health, PREDICT joined government partners in the Beni Regional Department of Health (SEDES Beni) and the Ministry of Public Health (MoH, Zoonoses, and Hantavirus/Hemorrhagic Fever Programs) to sample captured rodents in the affected area. Rodents were captured from agricultural fields and around houses, and samples were tested at the PREDICT partner lab (IBMB-UMSA) and confirmed by the UC Davis One Health laboratory. Response activities included rodent control measures and education/awareness campaigns. Joint efforts with SEDES technicians permitted testing biosafety measures on the ground and improved skills of field staff for capturing and sampling rodents during outbreak investigations.

The rabies outbreak in llamas in Potosi was believed locally to be associated with increasing attacks by foxes. The Bolivian government (MoH) and PAHO Bolivia invited SENASAG, the General Directorate of Biodiversity (DGB), and PREDICT to provide technical advice and expertise to help investigate and design a response strategy. Although local people were requesting fox culling, PREDICT promoted a preventive One Health approach: vaccination of both llamas and dogs and physical protection of llamas and livestock overnight with fencing. Culling of foxes

was discouraged to avoid ecological disruptions that could include, among other effects, massive increases in rodent populations and resulting damage to quinoa crops, the main livelihood of local people. Further studies carried out by PAHO and SENASAG showed that the rabies virus strain detected in the rabid fox was a domestic dog variant lending scientific evidence to PREDICT's outbreak response strategy.

PATHOGEN DETECTION AND CHARACTERIZATION

PREDICT Bolivia significantly enhanced early detection and quick characterization of yellow fever flaviviruses during a howler monkey mortality event near the Ambue Ari Wildlife Sanctuary Park (Alandia et al. 2013). The isolated viruses (strains TVP11767 and TN-96 NS5) were 94-97% identical to strains previously described in Trinidad and Tobago (Auguste et al. 2010) and from a person in Tennessee who contracted the disease in Brazil (Kuno et al. 1998), respectively. Preventive measures were rapidly implemented by the Bolivian government, and no known human cases occurred in the area of the outbreak (Alandia et al. 2013).

In addition to the detection of viruses currently undergoing further characterization, PREDICT enabled the detection of parasites and bacteria of zoonotic importance in Bolivian wildlife. *Echinococcus vogeli* was identified in an individual paca (*Cuniculus paca*; Gardner et al. 2013), while *Ascaris* sp. were detected in white-lipped peccaries (*T. pecari*; Limachi et al. 2014) hunted for consumption in a Tsimane indigenous community from the Pilon Lajas Biosphere Reserve. As part of a Public University of El Alto (UPEA) undergraduate veterinary thesis facilitated by PREDICT, *Salmonella enteritidis* and *S. typhimurium* were detected in parrots illegally sold as pets (Alarcon et al., unpublished data). Additionally, support provided to an UMSA masters student allowed the standardization of PCR protocols at the IITCUP laboratory and detection of *Chlamydia* spp. in confiscated parrots (Suárez et al., unpublished data).

ENSURING SUSTAINABILITY

To ensure sustainability of activities carried out by PREDICT in Bolivia from 2010 to 2013, efforts were made to increase the capacity of the Ministry of Health reference laboratories, CENETROP and INLASA, for detection of viral pathogens of zoonotic potential in animal samples. To this end, PREDICT in coordination with the National Program for Diseases Transmitted by Rodents and the IBMB PREDICT partner laboratory, donated laboratory protocols; 44 primers for detection of hantavirus, arenavirus, flavivirus, alphavirus, coronavirus, filovirus, and paramyxovirus; and two universal controls to CENETROP and INLASA in 2014.

Additionally, key PREDICT local partners (PCMB, EHA, former WCS/PREDICT veterinarians, INLASA, and IE) joined efforts to promote a One Health vision among

Indigenous technician Rosario Barradas (left) and veterinarian Fabiola Suárez (right) collecting bat samples at the Tuichi river in the San José de Uchupiamonas indigenous territory located inside the Madidi National Park.



PHOTO BY ERIKA ALANDIA, WCS



PHOTO BY ERICA ALANDIA WCS

PREDICT staff collect samples from a rodent during a survey conducted by the Museum of Natural History, Noel Kempff Mercado.

university students, human and animal health workers, and biodiversity authorities. This vision is now being applied to modeling of sylvatic rabies distribution for early warning of potential bovine cases in Northern La Paz, as well as for evaluating hantavirus activity in rodents from the San Buenaventura area where the first cases of hantavirus in the La Paz department were reported in 2012 (SEDES La Paz, 2012).

Similarly, former WCS/PREDICT veterinarians and the Museo de Historia Natural Noel Kempf Mercado presented a joint proposal to monitor hantavirus in an oil exploitation area from the Chapare Region (department of Cochabamba) where a fatal human case was reported earlier in 2014. Even though the project was not executed, the process enabled a collaboration agreement between the MoH's National Program for Diseases Transmitted by Rodents and the Museo de Historia Natural Noel Kempf Mercado for training of public health staff in rodent capture and sample collection methods. As a result, these institutions are currently implementing a hantavirus monitoring program in rodents in outbreak areas of Santa Cruz in collaboration with the CENETROP laboratory and partially funded by the Santa Cruz Municipality.

Furthermore, information of viral findings in bat samples provided by PREDICT to the Bolivian health authorities has induced the MoH SEDES La Paz to increase survey efforts in patients with respiratory syndromes coming from the areas where bats tested positive for corona, hanta, and influenza viruses. Moreover, a new laboratory facility to detect tropical diseases (including zoonosis such as leptospirosis, hantavirus, and yellow fever) was recently established in the Amazon region of the La Paz department, where PREDICT focused its wildlife monitoring efforts. This local lab will significantly improve efforts to prevent diseases in areas where great biodiversity and anthropogenic changes converge.

Continued interest in pursuing activities initiated by PREDICT through other external funding, in addition to enhanced diagnostic capacity at CENETROP and INLASA, illustrate PREDICT's positive impact in Bolivia through promoting science and local capacities to understand zoonotic disease ecology and a multidisciplinary team approach to the prevention of zoonotic disease outbreaks in the future.

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In Brazil, rapid economic development has led to deforestation, landscape fragmentation, agricultural and pastoral expansion, and human migration—all processes thought to influence zoonotic disease emergence.

BRAZIL

Brazil hosts 15-20% of the world's plant and animal diversity and the largest number of endemic species (Convention on Biological Diversity 2014). Nearly half of Brazil's geographic area is located within the single largest, continuous and least explored tropical rainforest, the Amazon. The Cerrado, Atlantic Rainforest, Caatinga, and Campos Sulinos complete a regional mosaic, which demonstrates this country's immense biological and ecological diversity. At the same time, Brazil has one of the largest and fastest growing economies. This rapid economic development translates into increased deforestation, landscape fragmentation, agricultural and pastoral expansion, and human migration; all processes thought to influence zoonotic disease emergence.

Within this context of change, it is notable that several cases of emerging and re-emerging infectious diseases have occurred in Brazil. For example, extractive industries in the Amazon region in the 1960s and 70s led to the discovery of new, potentially zoonotic arboviruses. These viruses were discovered in conjunction with highway construction in the Amazon and illustrate the increased risk of disease emergence associated with higher frequency of contact between vectors and hosts that harbor novel viruses. In order to better understand the risk of disease spillover and spread posed by human encroachment into pristine forests, research into the dynamic processes linking zoonotic disease emergence to human disturbance are needed, including studies investigating the diversity of pathogens and their ecology and epidemiology at the human-wildlife interface.

In Brazil, the PREDICT project aimed to evaluate how land-use change and other ecological factors drive disease emergence through alterations in biodiversity, corresponding viral diversity, and patterns of human abundance and behavior that may influence contact rates with wildlife.

FOR A SUMMARY OF THIS SECTION GO TO WWW.BRAZIL.PREDICT.GLOBAL

PREDICT conducted surveillance activities broadly across the country, including field sites in the Amazon, Cerrado, and Atlantic Rainforest. However, during the final years, the project focused on the Manaus region in Central Amazon.

Located at the heart of the Amazon region, Manaus has several features that make it an ideal location for the investigation of the ecology of infectious disease. The city is inhabited by 1.8 million people (Brazilian Federal Government 2014) and is surrounded by extensive areas of primary growth forest. Even within the city, small tracts of forest remain and support populations of wild animals. Manaus City has experienced several population bursts, including a boom in the 1960s for rubber plantations and influxes of immigrants drawn by the government-subsidized manufacturing industry. Sprawling urban development in Manaus, within the context of the surrounding Amazonian forests, provided a unique natural laboratory that had the potential to give new insight into disease ecology with both practical and theoretical implications.

PARTNERS

PREDICT Partners in Brazil included EcoHealth Alliance (EHA), Wildlife Conservation Society (WCS), USAID, Laboratory of Virology from the University of São Paulo (ICB), Solorio e Kautzmann, and the Center for Infection and Immunity at Columbia University (CII).

Other local partners included:

- Biosciences Institute, Laboratory of Epidemiology and Biostatistics (LEB); Laboratory of Compared Pathology (VPT) and Department of Veterinary Medicine, Prevention and Animal Health (VPS) of Faculty of Veterinary Medicine and Zoology (FMVZ), the University of São Paulo (USP)
- Sauim de Coleira Project
- Amazonian Federal University (UFAM)
- São Paulo Research Foundation (FAPESP)
- National Institute of the Amazon (INPA)
- Center for Screening of Wild Animals Manaus (CETAS/IBAMA)
- Mammals of Cerrado
- Biotropicos Institute
- Federal University of Minas Gerais (UFMG)
- University of Brasília (UNB)
- University of Santo Amaro (UNISA)
- Brazilian Institute of Environment and Natural Resources (IBAMA)
- Indigenous National Foundation (FUNAI)

Road construction and deforestation of one of the forest fragments that was home to a group of endangered pied tamarins.



PHOTO BY MONICA ROMERO SOLORIO

- Institute “Chico Mendes” of Conservation and Biodiversity (ICMBIO)
- Institute of Conservation Medicine (TRIADE)
- Ministry of Agriculture and Livestock (MAPA)
- National Foundation of Health (FUNASA)
- Amazônia Ocidental
- Institute for Research in Tropical Diseases of Rondonia
- National Center for Research and Conservation of Mammalian Carnivores (CENAP)
- National Center for Bird Conservation (CEMAVE)
- French Agricultural Research Centre for International Development (CIRAD)
- Universidade Federal de Pernambuco (UFRPE)
- Universidade do Vale do Itajaí (UNIVALI)
- Primate Refuge in Rio Grande do Sul State
- Mamirauá Institute of Sustainable Development
- Global Health Program, CDC
- US NAMRU-6 Lab, Lima Peru
- Authorization and Information System on Biodiversity (SISBIO)
- National Center for Research and Conservation of Brazilian Primates
- The Jungle Warfare Training Center of the Brazilian Army (CIGS)
- Manaus Center of Zoonosis Control (CCZ)
- “Emilio Goeldi” Museum
- Federal University of Pará (UFPA)
- Arca de Noe - Criadouro Conservacionista.

MAJOR ACHIEVEMENTS

- Executed the Deep Forest study to investigate how increasing land-use development affects biodiversity, viral diversity, and human distribution and behavior that may lead to the emergence of zoonotic diseases (see Success Stories for more information).
- Assisted a team of International Development Research Centre researchers with the Deep Forest Human Contact Survey to investigate contact between people and potential wildlife reservoir hosts.
- Developed a multidisciplinary network of collaborators among universities, government agencies, and non-governmental organizations across Brazil to conduct wildlife disease surveillance.

- Trained 46 people (48% women) in safe wildlife capture, handling, and sampling; surveillance; biosafety; biological specimen handling and shipment; and research ethics and responsibilities.
- Collected samples from 2,076 animals with approximately 85% of the samples coming from high-risk wildlife taxa (i.e. bats, rodents, and nonhuman primates).
- Conducted PCR analyses using PREDICT protocols for 24 viral families, genera, or species. The PREDICT laboratory partner, ICB, has the capacity to perform the PCR analyses using PREDICT protocols and to obtain sequence results from the PCR products.

SUCCESS STORY

Deep Forest

PREDICT developed a network of collaborators across Brazil. The team forged partnerships with Brazilian NGOs and agencies in order to establish a transdisciplinary team of conservationists, epidemiologists, and ecologists. The network assisted with surveillance across the country, illustrating the successful integration of PREDICT's One Health approach into the research activities of the scientific community in Brazil.

Project Development and Implementation. The aim of PREDICT in Brazil was to bring together a scientific team working toward One Health goals. This collaborative approach was exemplified by the Deep Forest study, which was developed to better understand the effect of landscape disturbance on viruses and their hosts and to create and strengthen ties to local scientific and stakeholder communities as part of capacity building and project development. These local partnerships provided a unique perspective, which allowed us to improve Deep Forest and better integrate it with the community. Brazil Deep Forest was developed through a collaboration between PREDICT researchers with expertise in mathematical modeling (modeling team) and local experts. Using baseline data and experience gained through PREDICT activities in Mexico, the Brazil team formally piloted Deep Forest in the third and fourth years of PREDICT implementation. During this pilot phase in Brazil, the PREDICT modeling team finalized the design and successfully extended the project to Uganda and Malaysian Borneo. The field methodology was established and refined through frequent discussions between local experts and the PREDICT modeling and Brazil field teams. These efforts led to a robust protocol that was based on landscape disturbance indices and was realistic given the varied local environments in the three countries.

Making a Difference for Conservation. During Deep Forest study, PREDICT-Brazil had frequent interactions with the community in Manaus. This interaction resulted in the guest invitation of our PREDICT Deep Forest partner, Solorio e Kautzmann, to participate in the “Plano de Ação Nacional” (PAN), a governmental organization composed of civilians who identify priority actions to combat threats to endangered populations of wildlife or natural environments. Since 2013, Solorio e Kautzmann has participated in the PAN committee



Dr. Catia Dejuste de Paula collects a sample from a bat in a remote and uninhabited area of the Brazilian Amazon.

for the highly endangered pied tamarin (*Saguinus bicolor*), the primary nonhuman primate species sampled in the Deep Forest study. The invitation was a direct result of how the team, in conjunction with other local partners, the Sauim de Coleira Project, PAN members, and local residents, spearheaded a successful conservation effort for a population of pied tamarins threatened by road construction in a local urban fragment. This effort increased the dialogue between the PREDICT team and local, national, and international media. Media highlights are available at:

http://acritica.uol.com.br/amazonia/Expansao-prejudica-especie-ameacada-amazonica_0_964703559.html

<http://banksiafilms.blogspot.com.br/2013/11/manaus-wildest-city-in-world.html>

<http://press.discovery.com/emea/apl/programs/worlds-wildest-cities-manauas/>

http://acritica.uol.com.br/amazonia/Expansao-urbana-prejudica-sauins-de-coleira-Manaus_5_964753516.html.

Lasting Change. Continued involvement of the team in PAN was a true testament to the success PREDICT had in building trust with the local community. In addition, the team cultivated a working relationship with CETAS/IBAMA, a governmental organization that protects the natural environment and oversees the sustainable use of natural resources. As PREDICT-Brazil built institutional bridges that solidified the team's influence in local affairs and conservation, it also broadened the ways in which the team impacted conservation and health in Manaus. For example, as a result of ties to local governmental organizations, the local Deep Forest team volunteered for a reforestation campaign designed to establish biological corridors connecting isolated urban fragments within the Manaus city limits. Through this effort, 700 fruit trees were



PHOTO BY PREDICT BRAZIL TEAM

The Brazil Deep Forest Team ready for a season of sampling.

planted in strategic locations to develop natural corridors for the pied tamarin. The PREDICT Deep Forest team supported this project to improve the utilization of One Health frameworks to solve local and global health and conservation issues.

CAPACITY BUILDING

Building a One Health Network

Members of the PREDICT Brazil team organized two symposia at the University of São Paulo, bringing together students, faculty, and researchers for lectures on One Health and viral emergence. Our Country Coordinator was invited by the Convention on Biological Diversity (CBD) and Pan American Health Organization (PAHO/WHO) to participate in the Regional Workshop on the Inter-Linkages between Human Health and Biodiversity in the Americas in Manaus, Brazil. The goal of this workshop was to support national efforts to introduce health issues into the National Biodiversity Strategies and Action Plans and to develop and update action plans to account for health and biodiversity. The workshop focused on national and regional levels to contribute to the Strategic Plan for Biodiversity 2011-2020 and the Aichi Biodiversity Targets.

In addition, several members of the team delivered oral and poster presentations to highlight the work conducted in Brazil at the Conferência Brasileira em Saúde Silvestre e Humana (Brazilian Conference on Wildlife and Human Health) sponsored by FioCruz in Rio de Janeiro and the Second International Congress on Pathogens at the Human Animal Interface (ICOPHAI) in Recife, Brazil. A publication on wildlife surveillance in the Amazon resulted from these presentations (Uhart et al. 2013).

Training

PREDICT-Brazil trained nearly 50 people in wildlife surveillance techniques and PCR and deep sequencing protocols. Trainees included postdoctoral, doctoral, and graduate students who conducted their projects within the PREDICT program, as well as local research scientists. One Brazilian postdoctoral fellow, who was trained at Columbia University in advanced molecular techniques and deep sequencing, went on to manage the diagnostic analyses performed at ICB for the PREDICT project.

Laboratory Infrastructure

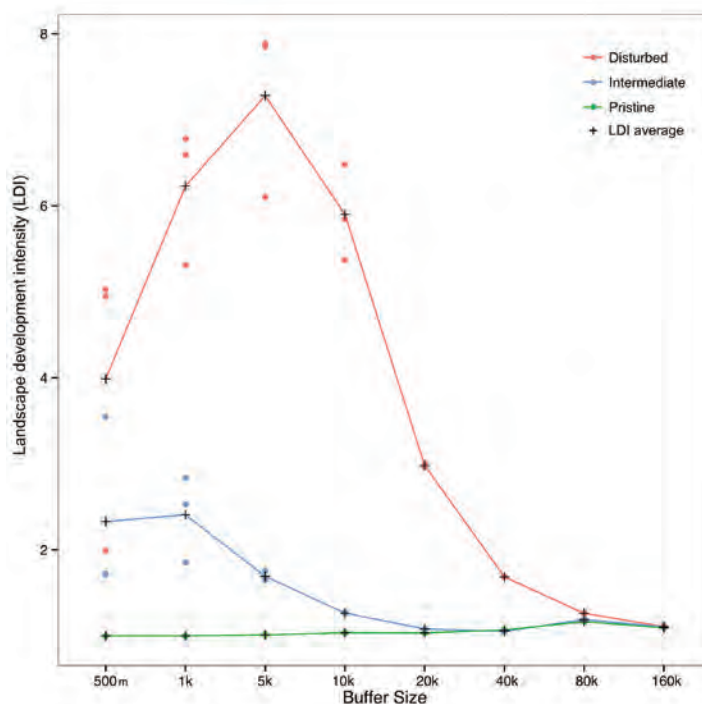
PREDICT also increased efficiency of testing at its partner laboratory, ICB, through improvements in infrastructure. ICB is a well-established BSL-3 laboratory, which had the capacity to conduct viral-family PCR analyses prior to PREDICT.

SURVEILLANCE

PREDICT collected samples from many species in several different biomes within Brazil. This included several trips to very isolated parts of the Amazon. In total, there were 75 field sampling events across the country. The surveillance was later targeted at high-risk taxa (i.e. bats, rodents, and nonhuman primates) in Manaus, Amazonas within the context of Deep Forest.

Deep Forest was designed to understand the interrelationships among biodiversity, emerging infectious diseases, and human ecology across areas of landscape disturbance from pristine forests to highly disturbed parks within the urban center. The PREDICT modeling and Brazil field teams used satellite imagery to develop a Landscape Disturbance Index (LDI; Figure 1) and used that data along with local knowledge to select the study sites.

Figure 1: Mean Landscape Disturbance Index values (across three replicate sites) for each of three levels of the land-use gradient (Pristine, Intermediate, and Disturbed) as a function of spatial scale.



Three study gradient levels were designated: the forest fragments in the City of Manaus (a highly disturbed and a densely populated city), Rio Preto da Eva (intermediately disturbed forest), and a protected area close to the BR-174 highway (pristine forest). A fourth fully converted agricultural site was also added to better describe the effects of disturbance on landscapes and was located in Rio Preto da Eva. Wildlife sampling was conducted during the rainy and dry seasons at three replicate sites in each of the landscape disturbance gradient-levels. In addition, surveys of human activities in the forest and frequency and type of contact with wildlife (see Deep Forest Human Contact Survey section below) were performed at each of the sites.

PREDICT-Brazil sampled a total of 2,076 animals (1,437 bats, 204 rodents, 66 nonhuman primates, and 369 animals from other taxa; Figures 2 and 3). This represents 88 bat species, 29 rodent species, 14 nonhuman primate species, and 35 species of other taxa. In order to focus the study in locations where spillover from wildlife to people may occur, the team focused their sampling activities on five primary human-animal interfaces representing behaviors or locations that are likely to result in human-wildlife contact and one interface where contact would be unlikely for reference. The interfaces with high-risk for disease transmission included wildlife in contact with park personnel, tourists, or researchers; wildlife in contact with workers in extractive industries (e.g. logging, mining); wildlife in and around human dwellings and agricultural fields; wildlife in urban forest fragments with recreational activities; and wildlife kept in private collections, such as zoos and sanctuaries, or as pets (Table 1).

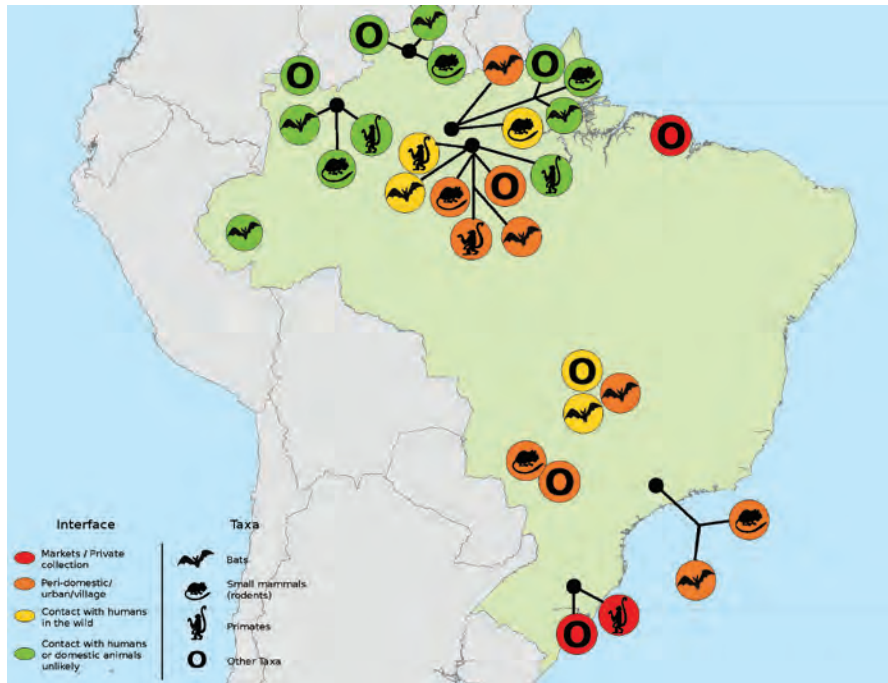


Figure 2. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

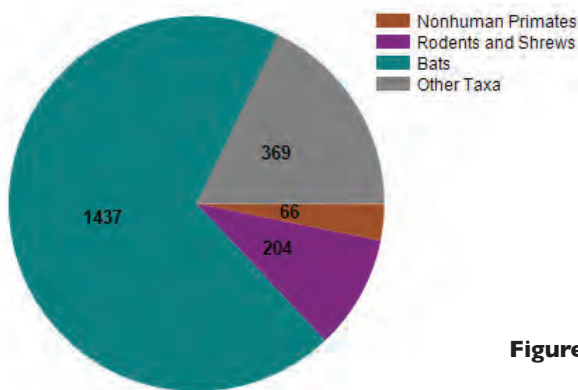


Figure 3. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	0	67	265	3
Ecotourism and recreational activities	8	0	107	12
Extractive industries	0	3	118	0
In or near human dwellings	18	76	415	189
Wildlife preying on livestock	0	1	0	1
Pristine habitat	8	53	340	105
Wildlife being studied	12	4	81	14
Protected areas	0	0	8	0
Zoos and sanctuaries	20	0	0	45
Other high-risk interfaces	0	0	103	0
Total:	66	204	1437	369

A total of 890 animals were sampled for Deep Forest (Table 2). In some parts of the Amazon, marsupials fill the niche of rodents and are more abundant. To better understand their potential as disease reservoirs and the risk that marsupials pose in the emergence of zoonotic pathogens, PREDICT-Brazil sampled marsupials and investigated contact rates between humans and these common animals.

Gradient	Taxa	Number
Pristine		
	Bats	108
	Rodents & Shrews	10
	Marsupials	30
Subtotal		148
Intermediate		
	Bats	334
	Nonhuman Primates	6
	Rodents & Shrews	70
	Marsupials	36
Subtotal		446
Disturbed		
	Bats	193
	Nonhuman Primates	37
	Rodents & Shrews	29
	Marsupials	37
Subtotal		296
Grand Total		890

Samples were analyzed using PCR at the partner laboratory, ICB, and deep sequencing was performed at Columbia University. In addition, the tamarin samples were processed in collaboration with the Laboratory of Epidemiology and Biostatistics, USP and the Sauim de Coleira Project, UFAM. Bats, rodents, nonhuman primates, and other taxa were tested for adenoviruses, alphaviruses, arenaviruses, astroviruses, bocaviruses, bunyaviruses, coronaviruses, enteroviruses, filoviruses, flaviviruses, hantaviruses, henipaviruses, herpesviruses, influenzas, lyssaviruses, Nipah viruses, orbiviruses, orthomyxoviruses, paramyxoviruses, parapoxviruses, phleboviruses, polyomaviruses, poxviruses, reoviruses, retrovirus - Lentivirus genus, rhabdoviruses, seadornaviruses, and

Table 2. Animals sampled under the PREDICT Deep Forest study by gradient and taxa from 2012-2014.

simian foamy viruses. Nonhuman primates were also tested for orthopoxviruses. Bird samples were tested for adenoviruses, arenaviruses, coronaviruses, filoviruses, flaviviruses, hantaviruses, henipaviruses, herpesviruses, influenzas, orthomyxoviruses, paramyxoviruses, retrovirus - Lentivirus genus, rhabdoviruses, and simian foamy viruses.

DEEP FOREST HUMAN CONTACT (DFHC) SURVEY

The DFHC survey investigated contact between people and potential wildlife reservoir hosts. Previous studies have shown correlations between frequency and type of exposure and risk of zoonotic infection. For example, people in frequent contact with nonhuman primates through their occupation, hunting, or keeping them as pets are at higher risk of infection with viruses harbored by nonhuman primates.

In zoonotic disease models, the dynamics of infectious diseases are dependent on the rate of transmission from infectious wildlife hosts to susceptible human hosts. To determine this component of risk, the rate at which people are coming into contact with potential wildlife hosts must be estimated. For known diseases, what constitutes a contact event will depend on the specific pathogen and its mode of transmission. However, for novel viruses this information is unknown.

For this reason, the chosen base unit of contact potential is any 'contact event' between humans and animals during which transmission could conceivably occur. The key element of understanding contact potential is thus to evaluate, quantify, and characterize human ecology via household surveys. The team's aim was to quantify and map patterns of human-animal contact across the land-use gradient to elucidate the relationships between specific land-use activities and contact. The quantification of human-animal contact rates, using an integrated approach, provides more accurate estimations of these variables in the model. This work was originally funded by International Development Research Centre (IDRC) but was an integral part of Deep Forest.

The IDRC anthropology team conducted focus groups, cognitive interviews, participant observation, and semi-structured interviews along the landscape disturbance gradient (in the same locations as the Deep Forest wildlife sampling). The IDRC team completed surveys in 618 households along the gradient (412 surveys at the urban site, 104 surveys at the pristine site, and 102 surveys at the intermediate site). Thus far, they have determined several uses of the forest areas by people, including, but not limited to laundering clothing in streams, recreating (e.g. children playing soccer), extracting fruit, and hunting. This IDRC data will be used in combination with the biodiversity and virodiversity data collected through PREDICT to better understand the effect of landscape disturbance and human ecology on the risk of zoonotic diseases.

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PHOTOS BY WILDLIFE CONSERVATION SOCIETY (LEFT) AND LUCY KEATTS, WCS (RIGHT)

The combination of rural poverty and the high demand for wild animal products locally and internationally, results in Cambodia being a key source, consumer, and conduit for wildlife trade.

CAMBODIA

The Kingdom of Cambodia is located in Southeast Asia, bordering Thailand, Lao PDR, and Vietnam and spans 181,035 km² (Kingdom of Cambodia 2010). Currently, the population exceeds 15 million, with a density of 82 people per square kilometer and with 6.3 million Cambodians existing on an income of less than two US Dollars per day. Greater than 30% of the population lives within five kilometers of forest, and forest resources account for 10% to 20% of household consumption supplies (FA 2010). The World Bank reports agriculture and forestry as contributing to nearly 40% of the country's Gross Domestic Product (GDP).

Cambodia is rich in biodiversity and forms part of the Indo-Burma biodiversity hotspot, having a high number of endemic species that are also threatened by the loss of a large proportion of their original habitat (Myers et al. 2000). Nearly 59% of Cambodia is still forested and includes evergreen, semi-evergreen, deciduous, swamp, mangroves, and bamboo forest. In the center of Cambodia is the ecologically rich Tonle Sap Lake that was named a UNESCO biosphere in 1997. Tonle Sap Lake is the largest freshwater lake in Southeast Asia. In the monsoon season, the lake increases significantly in size (Ministry of Environment 2001), and there is concurrent flooding of nearby fields and forests, providing important breeding grounds for fish and wetlands that provide habitat to migratory birds and a large variety of other wildlife.

Despite being listed as the 13th most forested country in the world (by percent land cover; FAO 2011), Cambodia's forests have been severely degraded over the past two decades as a result of logging, forest fires, intensified shifting cultivation, land-grabbing, and encroachment. Recent research has revealed that on a national scale, Cambodia was one of the worst three countries in terms of forest loss, with a loss of 7.1% of the country's forests from 2000-2012 (Hansen et al. 2013). Rapid economic development and granting of large land concessions for

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industrial agriculture and extractive industry have led to dramatic deforestation and large-scale land-use change. Few areas of Cambodia's forest remain untouched by anthropogenic disturbance (Figure 1). Such agricultural intensification and land-use change are key drivers for emerging diseases (Jones et al. 2008; Jones et al. 2013; MacFarlane et al. 2013), making Cambodia an important country of focus for disease surveillance at the increasing number of wildlife-livestock-human interfaces.

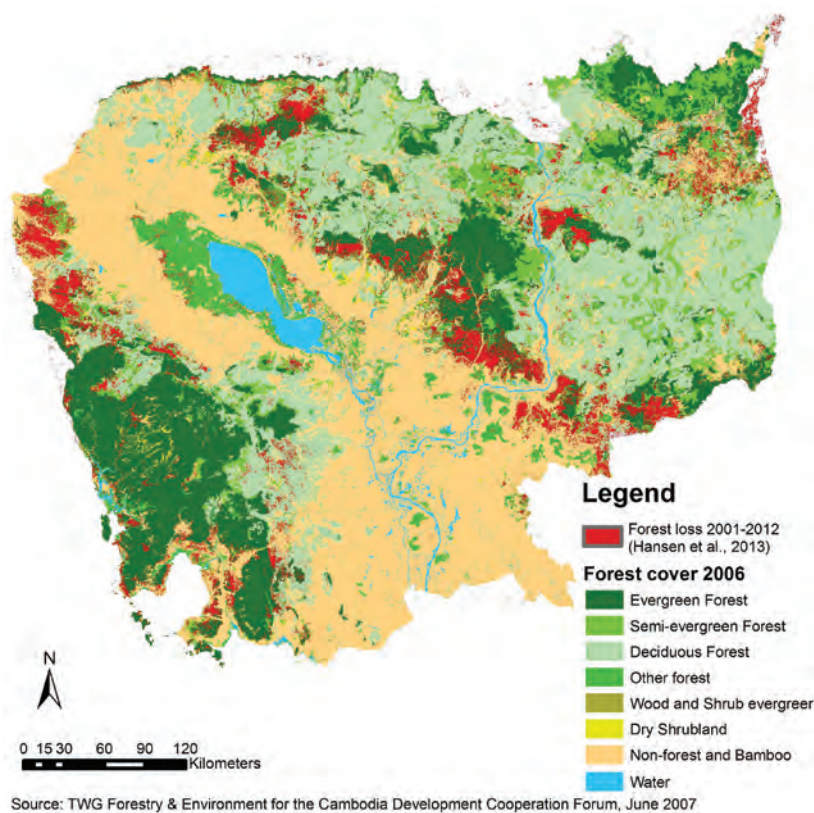


Figure 1. Map showing human disturbance of land across Cambodia.

There are currently 27 Protected Areas and 10 Protection Forests in Cambodia, representing about 24% of the country's total land area (UNEP-WCMC 2014). Existing forests provide a wealth of opportunities for wild game hunting, and the combination of rural poverty and the high demand for wild animal products locally and internationally (i.e. pet trade, food and traditional medicinal purposes), combined with porous international borders, results in Cambodia being a key source, consumer, and conduit for wildlife trade. Since 2001, the Forestry Administration's Wildlife Rapid Rescue Team has confiscated over 56,000 animals from the illegal wildlife trade in Cambodia (Wildlife Alliance 2014).

Bats and rodents are consumed and traded widely within Cambodia, and hunted nonhuman primates are traded for medical laboratory testing (macaques), traditional medicine (lorises), human consumption, and as pets (WCS, Cambodia unpublished). Seasonal trade results in over three tons of rats exiting Cambodia per day for consumption in Vietnam (Phnom Penh Post 2012). The scale and extent of global wildlife trade has expanded over the past decade. Increased purchasing power and globalization have led to a rising demand for exotic animals, many



Hunted Slow Loris (A small primate from the Genus *Nycticebus*), prepared for use in Khmer traditional medicine.

sourced from the wild, in both developed and developing nations (Nijman et al. 2012). Over 35 million CITES-listed animals were exported from Southeast Asian countries from 1998 to 2007 (Nijman 2010), and almost 30 million of these represented wild-caught individuals. These figures exclude all underground, local, and informal trade.

Since the discovery of SARS coronavirus in wildlife in Asian markets (Bell 2004), the wildlife trade is increasingly recognized as a potential source of zoonotic pathogen spillover and spread in human populations (Edmunds et al. 2011; Karesh et al. 2005; Pavlin et al. 2009; Smith et al. 2012; Swift et al. 2007). The scale of Cambodia's illegal wildlife trade makes it a high-risk country for the spillover

of emerging pathogens of wildlife-origin into humans and other animals. Early detection of potential zoonotic pathogens in wildlife is therefore important for prevention of emerging zoonoses in Cambodia and neighboring countries partnering in the wildlife trade. In Cambodia, PREDICT investigated the implications of human and wildlife interactions on risk of pathogen emergence, with a special focus on the wildlife trade.

Cambodia has recorded 56 cases of H5N1 Highly Pathogenic Avian Influenza infection in humans since 2005 and nine cases in 2014 as of March 17th (ProMED 2014). Most deaths have been linked to disease outbreaks in domestic poultry. With a history of inter-ministerial collaboration to respond to and combat the threat of avian influenza, the Royal Government of Cambodia recognizes the importance of a One Health approach to zoonotic disease surveillance, response, and prevention and the need to educate the next generation of animal and human health professionals in this cross-sectoral discipline.

PARTNERS

The PREDICT project was implemented in Cambodia through the collaborative efforts of Wildlife Conservation Society and the Pasteur Institute of Cambodia (IPC) to strengthen local capacity to conduct wildlife disease surveillance and to characterize zoonotic disease risk at wildlife-human interfaces. Other partners included USAID and:

Royal Government of Cambodia (RGC):

- Ministry of Agriculture, Forestry and Fisheries (MAFF)
- National Veterinary Research Institute (NaVRI)
- Forestry Administration (FA)
- Ministry of Health (MoH)
- Communicable Disease Control Department (CDC)
- Royal University of Agriculture (RUA)
- Prek Leap National College of Agriculture (PNCA)

MAJOR ACHIEVEMENTS

- Partnered with the Emerging Pandemic Threats PREVENT project to conduct a survey on wildlife hunting and consumption in several villages across three provinces. The aim of the survey was to assess zoonotic disease risk and design interventions targeted at high-risk behaviors (see Success Stories for more information).
- Institutionalized a One Health approach to surveillance, illustrated by a high-level of collaboration between government ministries and PREDICT, including agreements with the NaVRI and FA for their staff to join PREDICT field surveillance across 17 provinces (see Success Stories for more information).
- Established Khmer capacity for wildlife disease surveillance in the government and pre-service veterinary community through training of over 300 personnel (33% women) in PREDICT protocols and proper use of personal protective equipment (PPE). Collaboration among partners led to successful surveillance efforts across a variety of high-risk disease transmission interfaces from consumption of hunted wildlife in villages, markets, and restaurants to contact with wildlife in farms, tourist and religious sites, and rescue centers.
- In partnership with the IPC, PREDICT built in-country capacity for consensus (viral genus/family level) PCR and next-generation sequencing and provided training for NaVRI government laboratory technicians to ensure sustainability of the genomic tools.
- Engaged rangers and hunters from Khmer and ethnic minority villages in sampling and zoonotic disease risk determination activities across the country. PREDICT conducted 14 follow-up meetings in 11 provinces with communities involved in surveillance efforts to present project findings and discuss the risks of zoonotic diseases from wildlife. A total of 165 men and 248 women participated in the meetings. Local authorities concluded the meetings by advising villagers to minimize contact with wild animals through reducing high-risk hunting and trade activities and to improve their meat hygiene practices in order to reduce the risk of contracting diseases from wildlife.
- Participated as an invited member of the Zoonotic Technical Working Group, which includes RGC animal and human health sectors, WHO, and FAO. PREDICT contributed to the creation of the Strategic Plan for Zoonoses Control in Cambodia and worked with the RGC to move towards instituting a surveillance program for animals confiscated from the illegal wildlife trade.
- Integrated PREDICT into the higher-education community in Cambodia through participation of PNCA and RUA veterinary students in field surveillance activities and contributions to development of the new RUA veterinary doctorate degree curriculum.
- Test results from over 7,000 samples from 3,185 animals were presented to the Royal Government of Cambodia and approved for release to the public domain on PREDICT's HealthMap platform.

PHOTO BY WILDLIFE CONSERVATION SOCIETY



Inter-ministerial workshop on disease surveillance at the wildlife-human-livestock interface.

SUCCESS STORIES

Understanding Wildlife Consumption and Zoonoses Risk Perception in Khmer Communities

PREDICT and Emerging Pandemic Threats project, PREVENT, conducted a survey of wildlife hunting and consumption in Cambodia to assess potential risk of zoonotic disease spillover into people who participate in these activities. The survey was administered in nine villages across three provinces, concurrent with sampling of hunted wildlife. Hunting and consumption of wildlife are common throughout rural Cambodia, where many isolated, low-income communities still rely on wild animal meat as an important source of nutrition. Key animal groups identified as harboring the greatest proportion of zoonotic viruses (e.g. bats, nonhuman primates, and rodents) are still widely consumed in many villages. This survey aimed to quantify contact between wildlife and people and to begin to identify strategies to reduce the risk of spillover of zoonotic pathogens from wildlife into people since in the rural communities, minimal information is currently available to quantify seasonal household hunting or changes in the abundance and availability of wildlife.

Brief initial household surveys were conducted to obtain information about meat consumption and associated hunting and butchering activities to determine how potential risk associated with these activities was distributed among the population. Results of the household surveys were used to identify two groups of households:

1. Households who hunted and consumed the greatest number of animals in the previous month were invited to participate in the PREDICT sampling study and a quarterly follow-up survey about their domestic animals and consumption of meat; and
2. Comparison households, selected randomly from the other households in the village who agreed to participate in follow-up interviews, were invited to participate in the quarterly survey.

The surveys and surveillance were conducted in ethnic minority and Khmer villages in protected forest areas of Preah Vihear Province in northern Cambodia, Banteay Meanchey Province in Northwestern Cambodia, and in Mondulkiri Province in eastern Cambodia. Follow up quarterly surveys captured seasonal variation. These surveys identified key occupational and behavioral risks within the villages to target for PREDICT surveillance. In some provinces, over 8% of households consumed nonhuman primates, despite living within protected areas where promotion of the illegality of such activities was widespread and such consumption was not traditionally thought to be part of the local culture.

The study identified many reasons why people chose not to consume certain species of wildlife, including fear of disease, indicating some potential to influence and reduce local consumption through heightened awareness of health risks. However, such reasoning was observed to be very personal and not witnessed at an ethnic or community level, thus risk-awareness behavioral change approaches would likely be insufficient. Seasonality in hunting activities was observed, with many households turning to agricultural activities when the climate and landscape allowed, suggesting a preference to cease hunting when alternative foods and improved income sources were available.

The survey process allowed the team to build strong relationships with communities in villages within or bordering protected areas, many of which are now facing the prospect of large-scale local deforestation at recently granted Economic Land Concession sites. Continued surveillance

at these sites has great potential to capture information on the effect of land-use change on hunting patterns and pathogen emergence in wildlife and people.

Building a One Health Network for Surveillance of Zoonotic Pathogens in Wildlife

PREDICT worked closely with the RGC to build capacity for wildlife disease surveillance. The team in Cambodia held a workshop that brought together, for the first time, central and provincial staff from the Ministry of Agriculture, Forestry and Fisheries, MoH, Ministry of Environment (MoE), and higher education authorities. The workshop provided training on emerging zoonoses and surveillance in wildlife. The goals of this workshop were to highlight the cross-sectoral nature of this work and open communications for a One Health approach to collaboration across ministries in future wildlife disease surveillance efforts.

This workshop led to further training requests from the national veterinary schools and ranger teams working with confiscated wildlife. Additionally, the government recognized the importance of adopting the One Health approach, and PREDICT was invited to join the Zoonotic Technical Working Group meetings to bring a wildlife perspective to the discussions. Our field surveillance and diagnostics were a collaborative effort with technical staff members from the government's National Veterinary Research Institute and Forestry Administration as well as veterinary students who will become the future work force for disease response and surveillance.

CAPACITY BUILDING

Training

PREDICT provided training to a wide range of participants in order to build sustainable capacity for wildlife disease surveillance in Cambodia. Over 100 local partners were trained on core safety; wildlife capture and sampling; data collection and management; rodent, bat, and nonhuman primate identification; and proper use of PPE. In addition, training on wildlife zoonoses, safety, PPE, and sampling was conducted for rangers working closely with confiscated high-risk wildlife species (i.e. nonhuman primates, rodents, and bats). Furthermore, PREDICT held a workshop that brought together central and provincial staff from the MAFF, MoH, MoE, and higher education authorities for training on emerging zoonotic pathogens and wildlife disease surveillance and to promote collaboration across ministries.

PREDICT provided training to the next generation of Khmer veterinarians. A total of 187 students from PNCA and the RUA were educated on wildlife zoonoses. Twenty-two of these veterinary students also participated in field surveillance activities. PREDICT supported the completion of two veterinary thesis projects on nonhuman primate zoonoses, in which the students received training on field surveillance and laboratory analyses. In addition, RUA invited participation by PREDICT in a One Health workshop, which included a presentation on our surveillance strategy. Furthermore, RUA requested that PREDICT contribute to development of the new veterinary doctorate degree curriculum.

National Veterinary Research Institute staff member received training in PREDICT protocols and is teaching wildlife necropsy and sample collection techniques to the veterinary students.



PHOTO BY WILDLIFE CONSERVATION SOCIETY



PREDICT team member demonstrating use of liquid nitrogen for cold chain maintenance in the field.

Khmer language translations of the comprehensive PREDICT protocols were produced and disseminated to local partners, students, and ministries. Government staff and veterinary students have used the protocols to enhance wildlife disease surveillance in Cambodia through improved safety for humans and animals; heightened awareness of biosafety and ethical issues regarding both the treatment and handling of a variety of animal taxa during trapping, sample collection, or transportation; involvement of animals and humans in research; more appropriate sample and media selection; sample collection, storage, and handling techniques in the field and laboratory; and relevant data collection and management. Technical staff members from the NaVRI and the FA and veterinary students from the RUA and PNCA collaborated on wildlife disease surveillance activities.

Laboratory Capacity

PREDICT partnered with the IPC to introduce viral family PCR screening tools and next generation sequencing to Cambodia. PREDICT provided support for equipment and training to NaVRI government laboratory technicians at the IPC to ensure cross-sectoral sustainability of the diagnostic capacity.

SURVEILLANCE

Surveillance was conducted across 17 provinces covering all regions of the country and at 82 sites ranging from pristine protected forests to urban markets in populous areas (Figure 2). Country-wide surveys early in the project identified numerous interfaces that pose a high risk of spillover of pathogens from wild animals to humans. In addition, sampling at novel wildlife-human interfaces with high risk for disease transmission continued as the project progressed. The variety of interfaces that were targeted for PREDICT surveillance included (Table 1):

1. Wildlife hunting by ethnic minority and Khmer communities within and outside of protected areas;
2. Wildlife trade by middle-level traders operating within and outside of Cambodia;
3. Crop raiding by wild animals such as rats, wild pigs, and bats;
4. Zoos and wildlife rescue centers (e.g. Angkor Centre for Conservation and Biodiversity, the government wildlife rescue center at the public Phnom Tamao Zoo, and a private zoo where carnivores were fed macaques that had died on Khmer macaque farms);
5. Markets and restaurants selling wildlife (e.g. bat restaurants and markets selling primates for traditional Khmer medicine);
6. Bat guano farms where workers collect feces from under bat roosts for agricultural use;
7. Religious and ecotourism sites such as the Angkor Wat temple area, where macaques have been fed by the public and are now abundant and aggressive, and fruit bats roost amongst the temples and are sometimes targeted for hunting; and
8. Rodents in and around human dwellings and from the Mekong Delta region, traded to Vietnam for food.

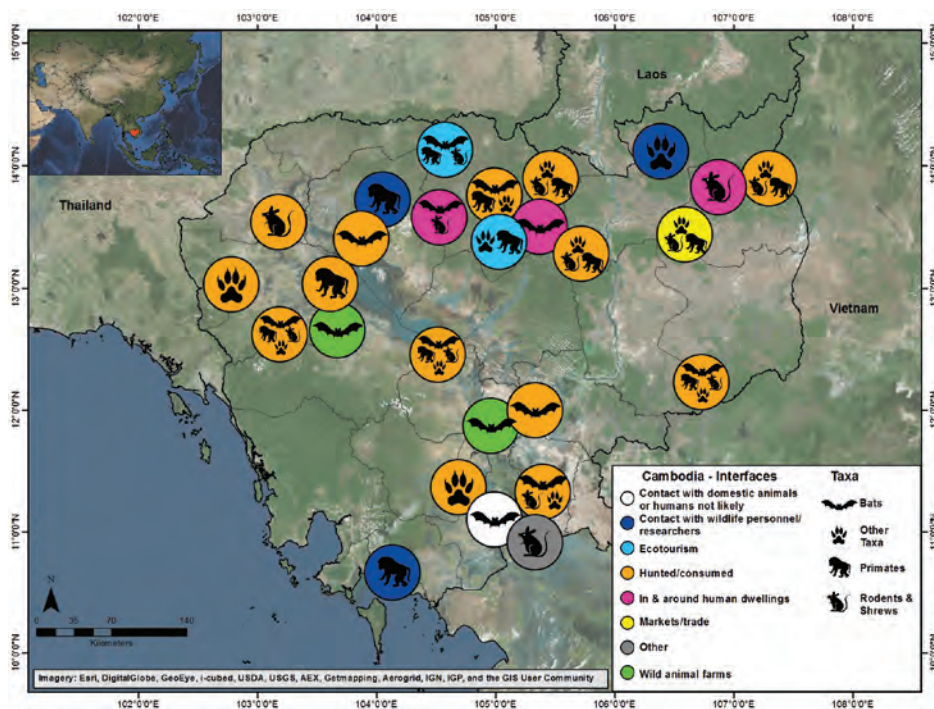


Figure 2. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	0	99	0	0
Ecotourism and recreational activities	41	10	96	22
In or near human dwellings	51	34	1041	0
Hunted wildlife	114	344	1017	394
Markets	54	11	0	115
Restaurants	0	0	347	0
Zoos and sanctuaries	40	0	0	1
Pristine habitat	0	0	2	0
Other high-risk interfaces	0	54	0	0
Total:	300	552	2503	532

A total of 3,887 animals were sampled including 300 nonhuman primates, 552 rodents, and 2,503 bats. Additional animal groups sampled included those hunted, consumed, and traded commonly by local people, such as wild pigs, deer, and civets (Figure 3). Polymerase Chain Reaction (PCR) screening for 16 viral families and additional pathogens of regional concern was conducted at IPC. The laboratory diagnostic testing occurred concurrent with training of local government veterinary laboratory counterparts, and in some cases veterinary students, to ensure sustainability of the diagnostic techniques in Cambodia.

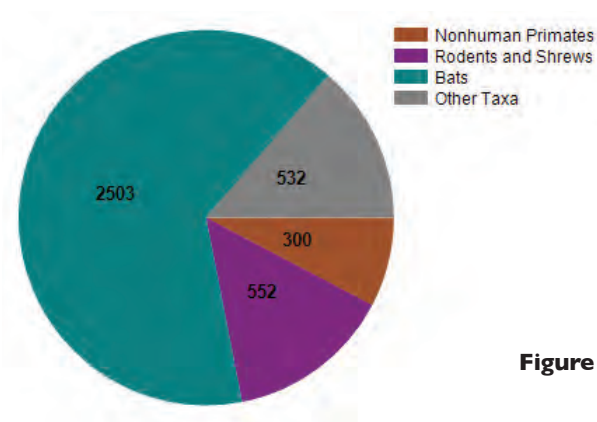


Figure 3. Number of animals sampled by taxa.

The following viral families were targeted for testing: arenaviruses, alphaviruses, astroviruses, bocaviruses, bunyaviruses, coronaviruses, enteroviruses, filoviruses, flaviviruses, influenza viruses, hantaviruses, henipaviruses, herpesviruses, orthopoxviruses, paramyxoviruses, rhabdoviruses, lyssaviruses, retroviruses (Lentivirus genus), simian foamy viruses, and seadornaviruses. In addition to optimizing viral screening protocols for wildlife samples and the introduction of two PREDICT universal controls to Cambodia, we initiated next generation sequencing at IPC for pathogen discovery.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

The focus on PREDICT surveillance concurrent with building of One Health capacity within the Royal Government of Cambodia and the future veterinary workforce has resulted in increased local technical capabilities and cross-sectoral communication to better respond to disease outbreaks involving wildlife.

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In Cameroon, hunting of wildlife is important for rural food security and livelihoods; however it is placing pressure on some wildlife populations, as the demand for bushmeat increases.

PHOTO BY MATTHEW LEBRETON, MB (LEFT) AND JOSEPH LE DOUX DIFFO, GV-C (RIGHT)

CAMEROON

Cameroon has a wide range of landscapes and climates, from dry Sahel regions, grasslands, and highlands to dense rain forests. With its varied geography and diverse habitats, Cameroon has a remarkable diversity and abundance of wildlife (Republique du Cameroun 2014). Agriculture is the mainstay of the economy, employing a large percentage of its rural workforce. In addition, timber extraction is undertaken across much of the forested areas in Cameroon, and mining is of increasing importance with a number of sites currently undergoing assessment or development for extractive activities. Cameroon is also an important transportation hub for goods going to the Central African Republic, Equatorial Guinea, Gabon, and Chad. Increased investment in major infrastructure projects, including transportation, water supply, and power generation is underway in areas that were once largely inaccessible.

Economic progress has led to advancing education levels and improved health, yet poverty remains high at 40%, and life expectancy is lower relative to the regional averages (World Bank 2014). Hunting of wildlife is a major cultural and economic activity in Cameroon – important for rural food security and livelihoods. However, it has placed extreme pressure on some wildlife populations, as urban and rural demand for bushmeat increases. Hunting and eating wildlife carries a substantial risk for cross-species transmission of zoonotic pathogens of wildlife origin.

Wildlife pathogens, including new retroviruses, have been detected in people that hunt and butcher wild animals in Cameroon (Wolfe et al. 2004, 2005; Zheng et al. 2010). Importantly, this high-risk activity in Central Africa was the likely pathway by which HIV emerged in people. Preventing the emergence of new infectious diseases and pandemics requires an enhanced understanding of the diversity of pathogens in wildlife, especially in areas with recent

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anthropogenic disturbance and high-risk human-wildlife contact, which increase the likelihood of emergence and spread of zoonotic pathogens.

In collaboration with the Government of Cameroon, PREDICT developed laboratory platforms and capacity to expand wildlife surveillance activities. The team conducted surveillance at high-risk human-wildlife interfaces in order to evaluate the diversity of known and novel viruses in wildlife that have the potential to spill over into people. In addition, project activities allowed for exploration of the implications of human and wildlife interactions on disease transmission and emergence. A focus on scientific collaboration and stakeholder and community engagement in Cameroon was key to our goals of increasing awareness of zoonotic disease risks, improving our understanding of disease emergence, and informing on potential mitigating measures to minimize the risk of cross-species transmission as well as rapid identification of viruses once they have crossed over into humans.

PARTNERS

PREDICT partners in Cameroon included:

- Ape Action Africa (AAA)
- Care and Health Program Cameroon (CHP)
- Centre Pasteur du Cameroun (CPC)
- Centre de Recherche de Sante des Armées/Army Health Research Centre (CRESAR)
- Global Viral Cameroon (GV-C)
- Limbe Wildlife Centre (LWC)
- Metabiota
- Ministère de la Defense/Ministry of Defence (MINDEF)
- Ministère de l'Environnement, de la Protection de la Nature et du Développement Durable/ Ministry of Environment, Protection of Nature and Sustainable Development (MINEPNDR)
- Ministère de l'Elevage, des Pêches et des Industries Animales/Ministry of Livestock, Fisheries and Animal Industries (MINEPIA)
- Ministère des Forêts et de la Faune/Ministry of Forestry and Wildlife (MINFOF)
- Ministère de la Recherche Scientifique et de l'Innovation/Ministry of Scientific Research and Innovation (MINRESI)
- Ministère de la Santé Publique/Ministry of Public Health (MINSANTE)
- Projet Grands Singes (PGS)
- Université de Douala/University of Douala (UD)
- Université des Montagnes (UDM)
- Université de Maroua/University of Maroua (UM)
- USAID



PREDICT provided community education on wildlife disease transmission risk, especially related to hunting and butchering of bushmeat.

non-clinical public health staff. These trainings were deployed to all 10 regions of Cameroon in September 2014 and were coupled with the dissemination of a national strategy for the fight against Ebola and specific surveillance and hospital management directives from the MoH. Educational materials were produced and displayed in strategic public areas, such as airports and hospitals.

- Enhanced disease surveillance capacity through training provided to staff from collaborating ministries and NGOs. This capacity building permitted standardization of protocols, introduction of novel techniques, and piloting of new technology as part of PREDICT (see Success Stories for more information).
- Collaborated with local communities to incorporate local knowledge into the surveillance strategy in Cameroon. Regular health education sessions were provided to enhance understanding among people who hunt and butcher wild animals of the risks associated with these activities and to share ideas for risk reduction (see Success Stories for more information).
- Implemented wildlife surveillance at high-risk disease transmission interfaces through a network of existing and new PREDICT partners, collecting specimens from 11,394 animals. Close collaboration with wildlife sanctuaries in Cameroon allowed regular sample collection from nonhuman primates originating from the wild or wildlife trade during animal health checks as well as during treatment quarantine periods.
- Augmented laboratory diagnostic capacity through laboratory infrastructure improvements and development of consensus and degenerate PCR assays to rapidly detect 20 viral families. Working molecular biology expertise was put in place locally to provide training and oversee laboratory analyses.
- Detected and characterized known and novel viruses in high-risk wildlife taxa and improved our understanding of their animal reservoirs and their potential for spillover into people (see Microbe Discovery and Characterization below for more information).

MAJOR ACHIEVEMENTS

- Provided key support to the Government of Cameroon in its initiative to develop the first National Program for the Fight against Emerging and Re-emerging Zoonotic Diseases. Development of this program enabled the inclusion of many actors in multisectoral discussions on the use of a One Health approach to control zoonotic diseases (see Success Stories for more information).
- Worked closely with the Directorate of Disease, Pandemic and Epidemic Control (DLMEP) of the Ministry of Public Health (MoH) to develop training materials around Ebola surveillance and control for clinical and

SUCCESS STORIES

Development of the National Program for the Fight against Emerging and Re-Emerging Zoonotic Diseases

PREDICT provided key support to the Government of Cameroon in its initiative to develop the first national program for zoonotic disease control. The development of this program enabled multi-sectoral discussions on zoonotic diseases and the One Health approach. For some participants, this interaction was the first time that they had been exposed to the One Health concept.

A National One Health Policy and National Program for the Fight against Emerging and Re-emerging Zoonotic Diseases plan were drafted under the leadership of the Prime Minister's Office and in collaboration with several ministerial departments and stakeholders, including hunting and conservation representatives. The plan was certified by four ministries, specifically the Ministry of Scientific Research and Innovation; the Ministry of Livestock, Fisheries and Animal Industries; the Ministry of Forestry and Wildlife; and the Ministry of Public Health. The ministries nominated points of contact for zoonotic diseases and One Health issues in order to develop a sustainable network of professionals for communication regarding One Health actions among ministries.

PREDICT staff provided guidance on field surveillance and laboratory diagnostics to ensure the program included innovations validated through PREDICT-Cameroon activities. The resulting program provided a sustainable framework that will guide and coordinate interventions among ministries and agencies with the goal of reducing zoonotic disease risks.

Building Capacity for a Sustainable Wildlife Surveillance Network

PREDICT's implementation required a significant effort to collect wildlife samples for virus detection from key high-risk interfaces. This approach presented unique training opportunities for staff and officials from collaborating ministries and the national veterinary laboratory. Participants in the training sessions included senior, central-level ministerial officials responsible for management and policy development, regional ministerial staff responsible for day-to-day management of wildlife conservation and veterinary services, and national veterinary laboratory staff responsible for laboratory diagnostic analyses.

PREDICT facilitated a comprehensive cycle of training for all participants that included theoretical training sessions on One Health, practical demonstrations of sample collection in the field, and laboratory analyses. This approach had multiple benefits. It provided an understanding by ministerial management staff of what is involved in wildlife surveillance activities, including the strategies behind the risk-based approach. It also allowed both local and central ministerial staff to see elements of surveillance in action. Furthermore, because there was already an understanding of the approach to surveillance and laboratory analyses by ministerial staff at the results reporting stage, discussions with collaborating ministries were focused on implications of the results and potential interventions to decrease risk.

Direct Community Engagement

PREDICT Cameroon strived to ensure its work was applied and a direct benefit to the local population. We collaborated with communities to ensure that there was an understanding by the members regarding the justifications for and techniques used in the project and to benefit from local knowledge in developing strategies for surveillance and risk reduction. Using this participatory approach, health education sessions were conducted regularly in order to inform individuals who hunt and butcher wild animals of the risks associated with this activity and to share ideas among community members of strategies to minimize these risks. The sessions allowed individuals to make informed choices about their hunting and butchering activities and provided them with feasible approaches to decrease their risk of exposure.

From 2010 to 2014, a total of 390 education sessions were conducted in 82 villages in the southern forest areas of Cameroon. This collaboration enabled the collection of over 23,000 dry blood spot samples from wild animals. As laboratory results from these samples became available, PREDICT staff shared relevant information with the communities in order to enhance their understanding of the risks associated with exposure during hunting and butchering of wildlife.

CAPACITY BUILDING

Capacity Building via PREDICT Staff

Due to the specialized and original nature of PREDICT activities, training was necessary to develop the relevant capacity among project staff and collaborators. Training sessions on zoonotic diseases allowed PREDICT and collaborating staff from ministries and NGOs to understand zoonotic disease risks associated with wildlife contact and how to minimize those risks through PPE and biosecurity. Other training included safe wildlife capture and sampling with proper techniques for restraint, handling, and safe sampling of wild animals. Additional sessions included information on proper specimen selection and handling and maintenance of a cold chain for sample storage and transport. Practical training on these principles took place on the job during field activities.

PREDICT provided training to government technicians to strengthen capacity for wildlife disease surveillance.



PHOTO BY JEAN MICHEL TAKUO, GVC

Major infrastructure improvements were also made, including installation of new laboratory equipment needed for pathogen discovery. PREDICT developed techniques that allowed detection of both known and unknown pathogens in samples collected in Cameroon. These techniques, using both consensus and degenerate primers with conventional PCR were fully implemented for detection of 20 viral families/genera: astroviruses, alphaviruses, arenaviruses, coronaviruses, filoviruses, flaviviruses, paramyxoviruses, seadornaviruses, hantaviruses, enteroviruses, rhabdoviruses, henipaviruses, bunyaviruses, influenza viruses, herpesviruses, bocaviruses, poxviruses, adenoviruses, polyomaviruses, and retroviruses.

Laboratory training sessions were conducted in order to provide participants with the skills to maintain a safe and biosecure laboratory environment. Proper use of PPE was also included in this training. For this component, staff from ministries and other collaborating organizations received PPE training and specific fit testing, using internationally standardized procedures to ensure the appropriate PPE size and fit for each participant. Personnel were also trained on laboratory database management. Four full-time PREDICT laboratory technicians were comprehensively trained in all steps of sample processing and techniques for pathogen discovery. In addition, molecular biology expertise was established locally for the duration of the PREDICT project.

To augment disease response capacity in Cameroon, PREDICT staff received training on zoonotic disease outbreak response. Additional training sessions included GIS mapping and disease modeling and information technology emergency management through the World Food Program.

Capacity Building for PREDICT Collaborators

Training opportunities, including short internships, were also provided to students from higher education institutions in Cameroon in order to promote laboratory competencies in the next generation of laboratory diagnosticians. Eight master degree students from the Higher Institute of Medical Technology (ISTM) Yaoundé completed a one-month internship, and 19 fifth year veterinary students from the Université des Montagnes attended a three day workshop in the PREDICT Cameroon laboratory. Students received training on One Health, wildlife sampling, laboratory techniques and biosafety, and disease outbreak response. Two students from the University of Yaoundé completed internships at the PREDICT-Cameroon laboratory as part of the Field Epidemiology and Laboratory Management Masters training program supported by CDC. In the course of the internship, they conducted an evaluation of the waste management system in the laboratory.

In addition, collaborators from Limbe Wildlife Sanctuary and Ape Action Africa were also trained on animal capture and sampling, biosafety, specimen storage and shipping, laboratory safety, first aid, and fire safety. Two university employees were also trained in the field on wild animal capture and sampling, included the use of traps and mist-nets, animal identification, animal restraint, and sample collection and storage.

Capacity Building for Cameroon Government Staff

PREDICT facilitated comprehensive training for staff and officials from collaborating ministries and the national veterinary laboratory. Participants received training on One Health, field surveillance, and laboratory methodology. This training provided ministerial management staff with an understanding of PREDICT surveillance strategies and viral detection methods, which facilitated more in-depth discussions of

PREDICT animal sample processing laboratory.



PHOTO BY METABIOTA



Animal capture and sampling training of staff from the Ministry of Livestock, Fisheries and Animal Industries and Ministry of Forestry and Wildlife by Cameroon PREDICT staff (bat identification).

results. In total, 40 ministerial staff including 24 from the Ministry of Forestry and Wildlife; 15 from the Ministry of Livestock Fisheries and Animal Industries; and one from the Ministry of Environment, Protection of Nature, and Sustainable Development participated in PREDICT trainings.

Two PREDICT Cameroon staff members presented laboratory approaches for detection of known and novel viral pathogens at the FAO meeting for national veterinary laboratory directors and Chief Veterinary Officers supported by the Emerging Pandemic Threats (EPT) IDENTIFY project. Among the participants were FAO/Identify staff from Cameroon, Italy, Mali, Kenya, and Botswana, as well as Chief Veterinary Officers and national veterinary lab directors from Cameroon, Congo, Gabon, Central African Republic, Democratic Republic of Congo, South Sudan, Uganda, and Tanzania. PREDICT also assisted the Cameroon-IDENTIFY team (WHO) and Ministry of Health in training 11 laboratory technicians from public hospitals in four regions of Cameroon on sample collection, packing, and shipping to reference laboratories for disease surveillance and outbreak response.

Regional Training Provided by PREDICT Cameroon

The PREDICT Cameroon laboratory provided field and laboratory training support to other PREDICT country programs in central Africa (Democratic Republic of Congo (DRC), Republic of Congo (RoC), and Gabon). PREDICT Cameroon staff travelled to DRC to provide training on GAINS, animal sampling, animal and human safety, laboratory safety, personal protective equipment (PPE), sample shipment, and ethical treatment of animals during capture and sampling. To advance capacity for wildlife pathogen screening, PREDICT Cameroon laboratory personnel provided materials for and training on protocols and implemented the laboratory analyses with the proper controls. PREDICT-DRC, Gabon, and RoC laboratory technicians also visited the PREDICT Cameroon laboratory for training on sample collection, processing, and testing; laboratory management, workflow, and high throughput analysis; and laboratory results interpretation.

In collaboration with PREVENT, PREDICT-Cameroon mapped and collected data on roadside and market bushmeat vendors in the Woleu-Ntem Province in Gabon in order to determine the logistics and feasibility of conducting market ethnography in the region. Staff also travelled to La Lope Reserve in Gabon for a USAID training implemented by the EPT RESPOND project and African Union – Inter-African Bureau for Animal Resources (AU-IBAR) on the topic of One Health for middle and senior staff from the Ministries of Health, Livestock, and Environment. PREDICT provided expertise and training on wildlife capture and sampling techniques to this group. To assist with the H7N9 outbreak in China, PREDICT Cameroon staff also participated in a capacity building and technology transfer visit to the partner laboratory in Guangdong, China.

SURVEILLANCE

A broad surveillance program was established in Cameroon in order to encompass the great diversity of high-risk human-wildlife interfaces, species, and landscapes in the country (Figure 1). The field team implemented the program through coordination with a network of partners, including government agencies, NGOs, and communities. Surveillance activities targeted areas where there was high risk for human contact with wildlife, especially nonhuman primates, bats, and rodents (Figure 2 and Table 1).

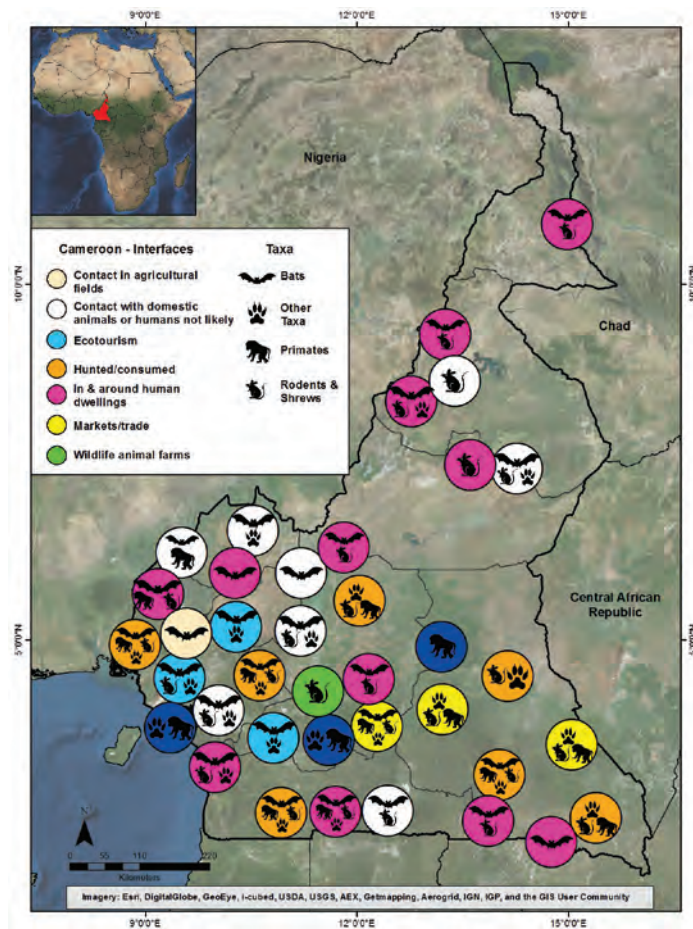


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

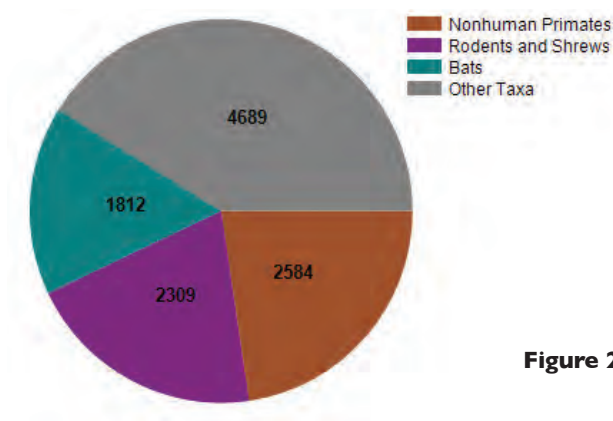


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	0	0	10	0
Ecotourism and recreational activities	0	35	136	11
In or near human dwellings	5	387	938	54
Hunted wildlife	1384	1770	596	4474
Wildlife being studied	1	0	0	0
Farmed wildlife	0	1	0	0
Zoos and sanctuaries	1028	0	0	19
Markets	25	62	0	64
Pristine habitat	141	54	113	67
Other high-risk interfaces	0	0	19	0
Total:	2584	2309	1812	4689

Surveillance sites were chosen based on their geographical distribution, the presence of high-risk wildlife taxa, and the risk of contact between people and the high-risk taxa. Sites with high-risk disease transmission interfaces were identified through expert technical advice and local community knowledge.

Three main types of sites were selected for surveillance activities:

- Sites in the southern forested area of the country where many people rely on forest resources for income generation and subsistence, including bushmeat hunting to supplement farming activities. PREDICT activities in these areas included educating hunters about reducing their risk of contracting zoonotic diseases and wildlife conservation.
- Sites in the Mount Cameroon area and savanna in northern Cameroon where there is a growing ecotourism industry and increased opportunities for contact between tourists and wildlife. These sites have diverse fauna that is distinct from the fauna in the southern forested areas. The landscape of these sites shares similarities with that of the area in Nigeria where the Lassa virus outbreak occurred.

- Sites throughout Cameroon where peridomestic settings were targeted. Rodents and bats live in and around human dwellings and agricultural fields where they may come into direct or indirect contact with people.

A number of different collaborations facilitated surveillance activities. Wildlife samples were collected in partnership with people hunting and butchering wild animals at markets and in villages. While harvested wildlife provides an important source of protein in the daily diet of many villagers in Cameroon, the harvesting rate is not always sustainable. Therefore the field team, accompanied by Ministry of Wildlife officials, sought to reinforce community knowledge of protected species in an effort to reduce the harvesting of these species. In addition, collaborations with wildlife sanctuaries (Limbe Wildlife Sanctuary and Ape Action Africa) allowed regular sampling of nonhuman primates that originated from the wild or the wildlife trade. Furthermore, people holding nonhuman primates in private collections as pets provided samples.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

Participation in the Ministry of Health Surveillance Meetings

PREDICT staff participated in weekly disease surveillance coordination meetings at the Department of Disease Control at the Ministry of Health. The purpose of these meetings was to follow-up on epidemics occurring at the national and international levels and to discuss control strategies. These meetings provided opportunities to build upon One Health networks and establish PREDICT surveillance strategies that are adaptive and integrated across the animal and human health sectors.

Deployable Disease Outbreak Response Team

To enhance preparedness for disease outbreak response, PREDICT Cameroon organized a deployable outbreak response team to assist with wildlife investigations during an outbreak. A series of response plans were developed, and capacity was established to screen for a range of pathogens in the field, including influenza A and B, Ebola virus, yellow fever, Lassa virus, dengue virus, and primate T lymphotropic virus 3/4.

The team informed the Ministries of Health and Livestock on arenavirus animal surveillance activities undertaken by PREDICT during the outbreak of Lassa fever in Nigeria where 40 people died, and 400 people were diagnosed with the disease. The team's activities were discussed with the Minister of Livestock and the Secretary of State for Pandemics and Epidemics at the Ministry of Public Health. At the suggestion of the Secretary of State, a request was submitted to the Ministry of Public Health to integrate the PREDICT Cameroon team into the epidemic response working group. A memo describing the outbreak response capacities of the PREDICT partner in Cameroon was submitted with this request.

DRC Outbreak Support

Two PREDICT Cameroon staff travelled to DRC in September 2012 to assist with the Ebola outbreak in Isiro (Haut Uele Province). PREDICT provided technical support for wildlife surveillance activities conducted by district personnel from the Ministry of Environment, Nature Conservation and Tourism and the Ministry of Agriculture and Livestock. A daily report was given to the International Committee on Scientific and Technical Coordination for Fighting



Bat sampling during Ebola virus outbreak in DRC.

Against Ebola Hemorrhagic Fever Virus in the district of Haut Uele under the Lab and Research sub-committee.

Three wildlife sampling strategies were implemented during the outbreak. Free-ranging wildlife in and around Isiro and surrounding villages were captured and sampled. In addition, pets and hunted wildlife in these areas were sampled. Samples were collected from 78 bats from 10 species, including two species known to be involved in the ecology of Zaire ebolavirus (*Epomops franqueti* and *Hypsignatus monstrosus*). Information gathered by PREDICT in Isiro during the visit indicated that hunting and consumption of bats, in particular *Hypsignatus monstrosus* and *Epomops franquetii* bats, occurs locally. In addition, the team travelled to the nearby towns of Nalamu and Xavierou to investigate reports of animal carcasses in the forest. They found cases of bushmeat for sale, despite current

regulations against hunting in the area. Samples were collected from the hunted animals and transferred to the PREDICT / INRB laboratory in Kinshasa for testing.

Monkeypox Outbreak in Chimpanzees

PREDICT assisted the Ministry of Health, Ministry of Forestry and Wildlife, and Ministry of Livestock, Fisheries and Animal Production in responding to the first confirmed outbreak of Monkeypox in wildlife in Cameroon. In July 2014, the Ministry of Health was notified of chimpanzees with symptoms suggestive of a monkeypox outbreak in the Sanaga Yong Chimpanzee Rescue Centre. PREDICT assisted the government in the field investigation and confirmed the Monkeypox diagnosis in the PREDICT laboratory in collaboration with CDC. Following the notification of results to the sanctuary and the government, the Chief Veterinary Officer declared the outbreak via the OIE and undertook a follow-up site visit to ensure preventive measures recommended during the initial visit were in place and that the infections were not spreading.

Ebola Outbreak Preparedness

PREDICT-Cameroon worked closely with the Directorate of Disease, Pandemic and Epidemic Control (DLMEP) of the MoH to develop training materials for Ebola surveillance and control for clinical and non-clinical public health staff. Trainings were deployed to all 10 regions of Cameroon in September 2014 in conjunction with the dissemination of a national strategy for the fight against Ebola and specific surveillance and hospital management directives from the MoH. Educational materials were developed and displayed in targeted public places, including airports and hospitals. We also assisted the Ministry of Livestock in drafting an Ebola surveillance plan for wildlife in Cameroon and contributed to workshops to discuss priorities and the role of regional Ministry of Livestock staff.

MICROBE DISCOVERY, CHARACTERIZATION, AND CROSS-SPECIES TRANSMISSION

PREDICT built upon previous long-standing research collaborations with rural populations, wildlife sanctuaries, and government agencies in Cameroon to further investigate the potential for spillover of pathogens from nonhuman primates to people through a number of studies, including:

- **Evidence for Henipavirus Spillover into Human Populations in Africa.** Zoonotic transmission of lethal henipaviruses (HNVs) from their natural fruit bat reservoirs to humans has only been reported in Australia and South/Southeast Asia. However, one study discovered numerous HNV clades in African bat samples. To determine the potential for HNV spillover events among humans in Africa, PREDICT examined sets of bat and human serum samples from Cameroon for Nipah virus cross-neutralizing antibodies. We detected antibodies in 48% and 3-4% of the bat and human samples, respectively. Seropositive human samples were found almost exclusively in individuals who reported butchering bats for bushmeat. Butchering bat meat and living in areas undergoing deforestation were the most significant risk factors associated with seropositivity (Pernet et al. in press).
- **A Gorilla Reservoir for Human T-lymphotropic Virus Type 4 (HTLV-4).** This research identified gorillas as a nonhuman primate host for HTLV-4 (LeBreton et al. 2014). These findings highlight the diversity of retroviruses being transmitted to people via the same pathway used by HIV in its emergence (LeBreton et al. 2014).
- **Widespread Infection with Homologues of Human Parvoviruses B19, PARV4, and Human Bocavirus of Chimpanzees and Gorillas in the Wild.** This research showed that the nonhuman primate homologues of the three human parvoviruses circulate extensively among wild ape populations with particular high rates of exposure in chimpanzees (Sharp et al. 2010). Further characterization of PARV4-like viruses in chimpanzees and colobus monkeys revealed that these viruses appear to be species-specific, despite vast opportunities for cross-species transmission, suggesting that the risk of spillover of PARV4-like viruses from nonhuman primates into humans is low (Adlhoeh et al. 2012).
- **Species Association of Hepatitis B virus (HBV) in Nonhuman Apes – Evidence for Recombination between Gorilla and Chimpanzee Variants.** PREDICT investigated species-specificity of hepatitis B virus (HBV) among nonhuman primate species and between humans and nonhuman primates using complete genome sequencing and phylogenetic analysis (Lyons et al. 2012). Results of this study provide the first evidence for HBV circulation between chimpanzees and gorillas and among subspecies of chimpanzees, a conclusion that differs from the dogma of strict host specificity of HBV genotypes, suggesting that there is potential for spillover (and the emergence of new genotypes) of this virus into new species, including humans (Lyons et al. 2012).
- **High Seroprevalence of Enterovirus Infections in Apes and Old World Monkeys.** Enterovirus species A, B, and D were detected in wild chimpanzees. The potential for spillover and spread of enteroviruses from old world monkeys or apes to humans is unknown; however, evidence of enterovirus circulation among these nonhuman primates warrants assessment of potential sources for the periodic emergence of novel enteroviruses (Harvala et al. 2012).

- **Characterization of a New Simian Immunodeficiency Virus Strain in a Naturally Infected *Pan troglodytes* Chimpanzee with AIDS-related Symptoms.** PREDICT characterized a new simian immunodeficiency virus strain in a naturally infected *Pan troglodytes troglodytes* chimpanzee with AIDS related symptoms, the reservoir of the ancestors of HIV-1 in humans, (Etienne et al. 2011). This research revealed that SIV has an increasing viral diversity over time and provided further evidence that chimpanzees can have clinical progression to an AIDS-like disease (Etienne et al. 2011).
- **Failure to Detect Chronic Simian Immunodeficiency Virus in a Large Cameroonian Cohort with High Nonhuman Primate Exposure.** PREDICT assessed simian immunodeficiency virus (SIV) infection in individuals who hunt and butcher nonhuman primates in Cameroon, a population in which other nonhuman primate retroviruses were previously detected. Some individuals had evidence of previous exposure, but nucleic acid sequences of SIV genes could not be detected, suggesting that SIV infection in humans could occur less frequently than infections with other retroviruses (Djoko et al. 2012).
- **African Origin of *Plasmodium vivax*.** PREDICT researchers also participated in a study investigating the origins of the malaria parasite, *Plasmodium vivax*. Results of this research indicate that *P. vivax* is of African and not Asian origin and that all extant human parasites evolved from a single ancestor that spread out of Africa. The high prevalence of *P. vivax* in wild-living apes has implications for malaria eradication efforts (Liu 2014).

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Industrial and small-holder poultry farms, in combination with the wildlife trade, are growing to meet increasing food demands in the country.

PHOTOS BY ALEKSEI CHMURA, EHA (LEFT) AND BY PETER DASZAK, EHA (RIGHT)

CHINA

China is home to the most endemic temperate biodiversity of fauna and contains approximately one-tenth of all known species. More than 4,000 vertebrate species live within the borders of China, and changes in land-use are rapidly altering these animals' natural environments. Some of China's most biodiverse areas – in South Guangdong, Guangxi, Hainan, and Yunnan Provinces – are also among its most populous. China's population is the largest in the world, and the country is undergoing the greatest rate of development (Seto et al. 2000). Demographic changes are yielding a rapid centralization of the population in expanding urban centers accompanied by growing demands for resources, including food (Peng 2011). Extraction of natural resources and shifts in agricultural practices and intensity to meet food demands have resulted in large-scale changes to China's landscape, especially in southern China where rice and domestic animal production are greatest (Seto et al. 2000). In addition to the growing industrial and small-holder poultry farms located throughout urban and rural China (Rae 2008), the country also significantly contributes to the wildlife trade for food, pets, and luxury items, sourcing products through in-country wildlife farming and importation from around the world.

As one of the largest and most geographically diverse countries with a rapidly developing economy, China is a key player in global wildlife trade and transport with potential concomitant impacts on health and the environment. Countries in Southeast Asia are rich in wildlife biodiversity (Myers et al. 2000) and have experienced some of the most rapid deforestation, bringing human settlements and commercial agriculture into proximity with wildlife (Achard et al. 2002). In addition, illegal wildlife trade is pervasive in Southeast Asian countries, including China, Malaysia, and Thailand (Nijman 2010). Innumerable species are shipped from Southeast

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Asia and southern China throughout China, as demand for wild animals increases with per capita income (Nijman 2010). The wildlife trade played a role in the emergence of SARS, avian influenza, and other diseases of wildlife origin (Karesh et al. 2005; Smith et al. 2012).

Guangdong Province in China is where the first cases of SARS were reported in 2002. Now known to have originated in bats (Li et al. 2005), SARS emerged in humans and other mammals in live animal markets (Guan et al. 2003). The disease spread to 26 countries and infected over 8,000 people; killing nearly 800 (Stadler et al. 2003). SARS shut down trade of domestic animals and resulted in upwards of \$30 billion in losses to the global economy. Subsequent outbreaks of H5N1 and more recently H7N9 in 2013 and 2014 demonstrated the realities of the risk of disease transmission and spread in China and the detriments to animal and human health, as well as human livelihoods (Smith et al. 2006; Gao et al. 2013).

The increasing frequency and variety of human-wildlife interactions in China present opportunities for zoonotic disease transmission (Karesh et al. 2005). For example, handling or consumption of hunted or farmed wildlife poses a risk of pathogen spillover into humans (Wolfe et al. 2005). Besides home consumption, in southern China provinces like Guangdong, increasing numbers of individuals are traveling to urban-rural interface areas to eat at restaurants, where many hunted or farmed wildlife are kept alive. In addition, the intermingling of wildlife in wet markets can lead to inter-species transmission of pathogens and may result in spillover into a species that can more efficiently transmit the pathogen to humans (Bengis et al. 2004). China's rich diversity of industry and culture also present human-animal interfaces through activities such as ecotourism, religious activities, natural resource extraction, and wildlife-human conflict situations (e.g. crop-raiding; Karesh et al. 2005; Chomel et al. 2007). Proactive surveillance of wildlife at these and other high-risk human-animal interfaces is needed to identify zoonotic disease risks for humans.

In China, we aimed to detect, track, and predict the emergence of new infectious diseases in high-risk wildlife taxa that could pose a major threat to human health. PREDICT's work in China was critical because the diversity of potentially zoonotic pathogens hosted by wildlife is likely to be commensurate with the diversity of wildlife, thus putting China at high risk for zoonotic disease outbreaks. PREDICT increased capacity for a One Health approach to disease outbreak response in China. The program implemented surveillance in high-risk human and animal populations, as well as hospital syndromic surveillance for fevers of unknown origin, in partnership with provincial and local centers for disease control (CDCs) under the Ministry of Health. Particular focus was placed on establishing an enhanced wildlife monitoring capacity in geographic "hotspots" in southern China that pose a particular risk for the emergence of novel infectious diseases.

Bats, doves, chickens, ducks, and other birds in cages in a live animal market in Guangdong province.



PHOTO BY ECOHEALTH ALLIANCE

Wildlife disease surveillance focused on targeted discovery of specific priority viruses, including retroviruses, influenza, coronaviruses, and filoviruses in wildlife sampled in natural habitats and the market trade and from wildlife products and foods. Human disease surveillance targeted individuals with high levels of exposure to wildlife (e.g. butchers) and followed them over time to determine their exposure to zoonotic pathogens. In addition, PREDICT conducted syndromic surveillance by applying PREDICT diagnostic protocols to specimens from individuals with key symptomology (e.g. fever, diarrhea, encephalitis). PREDICT China's in-country partner laboratories focused on virus discovery, especially viruses causing outbreaks of disease and identifying new potential pathogens of pandemic risk. Surveillance and ecological data in combination with GIS information were used to assess the risk of emerging diseases and evaluate mitigation strategies for decreasing risk of pathogen spillover from animals into people in China.

PARTNERS

PREDICT partners and colleagues in China have well-established facilities including infectious disease diagnostics labs with state-of-the-art molecular virology and serology capacity. Dr. Shuyi Zhang, based at East China Normal University, was the PREDICT China Country Coordinator, and has extensive experience working with wildlife and leading investigations of viral diversity in wildlife. He was part of the original investigation team that identified bats as reservoirs for SARS CoV. PREDICT's partners at Guangdong CDC were at the epicenter of the SARS outbreak, and developed surveillance programs with PREDICT using a One Health framework.

Because of the strengths of its partners, the China PREDICT team had unparalleled access to wildlife trade systems in China spanning all levels of the trade network from hunters in source areas, through conduit routes, markets, consumers, illegal wildlife restaurants, and wildlife department holding facilities (confiscated wildlife). PREDICT was able to obtain detailed information about hunting behavior and work with provincial CDCs to screen blood samples alongside the hunted animal samples for zoonotic pathogens.

**Cormorant fishing
at Erhai Lake,
Yunnan, China.**



PHOTO BY PETER DASZAK, EHA

In China, PREDICT partnered with USAID and set up long-term collaborations with the Wuhan Institute of Virology (WIV), the Yunnan Institute of Endemic Disease Control and Prevention, Guangxi Normal University, Shanghai Municipal Center for Disease Control and Prevention (SHCDC), the Guangdong Provincial Center for Disease Control and Prevention (GDCDC), the Guangdong Entomological Institute (GDEI), and East China Normal University (ECNU). In addition, PREDICT developed a collaborative relationship with Guangdong Institute of Public Health (GDIPH)/GDCDC, which also bridged joint works with Guangdong Zhanjiang Ratproof Institution. Further, PREDICT developed and maintained closely collaborative relationships with prefecture level CDCs that are located in Fengkai (Zhaoqing), Deqing (Zhaoqing), Yunfu, Yunan (Yunfu), Dabu (Meizhou), Jiaoling (Meizhou), Pingyuan (Meizhou), Xinyi (Maoming), Heping (Heyuan), Lianping (Heyuan), Lianshan (Qingyuan), Lianzhou (Qingyuan), Shaoguan, Zhanjiang, Dongguan, Jiangmen, Huizhou, Zhongshan, Luoding, and Yangjiang.

Other than local CDCs, PREDICT established long-term collaborative relationships with sentinel hospitals located within Guangdong Province. These included Gaozhou People's Hospital, Maoming; Yunfu People's Hospital, Yunfu; Luoding People's Hospital, Yunfu; Deqing People's Hospital, Zhaoqing; Fengkai People's Hospital, Zhaoqing; Donghua Hospital, Dongguan; Dongyuan Traditional Chinese Medicine Hospital, Heyuan; Lianping People's Hospital, Heyuan; Meizhou People's Hospital, Meizhou; Shaoguan Yue Bei People's Hospital; Zhanjiang Central People's Hospital; Jiangmen Central Hospital; Huizhou First People's Hospital; Zhongshan People's Hospital; Maoming People's Hospital; and Yangjiang People's Hospital. In 2013, an EPT PREDICT-GDIPH/GDCDC collaboration facilitated viral pathogen identification through interprovincial joint activity with Jiangsu Provincial Center for Disease Control and Prevention (JSCDC).

MAJOR ACHIEVEMENTS

- Promoted a One Health approach to human disease surveillance, enhanced laboratory testing capacity, improved inter-sectoral communication, and more broadly facilitated detection of cross-species viral sharing during the H7N9 outbreak. PREDICT tested a total of 167 samples from people with influenza-like illness in addition to 27 environmental samples. The laboratory team implemented protocols developed by PREDICT to expand detection of divergent strains and to improve sequencing capability (see Success Stories for more information).
- Isolated SARS-like coronaviruses from bats for the first time in China and fully characterized the whole genome of two novel bat SARS-like coronaviruses (see Success Stories for more information).
- Implemented PREDICT protocols in a federal virology diagnostic institute (WIV) for viral discovery and at the Guangdong Provincial Center for Disease Control and Prevention (GDCDC) as a diagnostic tool for hospital-based surveillance specimens.
- Improved wildlife field surveillance and diagnostic testing capacity in multiple institutions. Optimized PREDICT surveillance protocols by developing an animal field guide and providing training for all collaborating institutions and individuals.
- Over 3,061 bats, 737 rodents and shrews, and 146 other animals were sampled in animal markets, farms, and rural areas. A total of 1,267 humans were sampled at human-animal interfaces, coupled with animal sampling.
- Enhanced communication among ministries focused on infectious diseases and initiated conversations concerning future collaboration among ministries, including data and sample sharing, as well as identification of new strategies for cooperative research.
- Generated interest among regional Centers for Disease Control in testing samples for plague (rodents) and rabies virus (bats), as well as other zoonotic viruses, using PREDICT protocols. This expansion of testing leveraged existing collection activities to expand wildlife surveillance regionally.
- Identified live animal markets along wildlife trade routes in Southern China, where novel diseases are likely to emerge.

**Analyzing
Coronavirus
diversity at the
Wuhan Institute
of Virology.**



PHOTO BY ZHENGLI SHI, WUHAN
INSTITUTE OF VIROLOGY

- Conducted a Guangdong Hospital syndromic surveillance study using PREDICT protocols. Patients exhibiting encephalitis symptoms were tested for seadornavirus, flavivirus, paramyxovirus, hantavirus, and arenavirus. Moreover, samples collected from individuals with hemorrhagic fever, or fever with thrombocytopenia, were screened for paramyxovirus, arenavirus, hantavirus, filovirus, and flavivirus.

SUCCESS STORIES

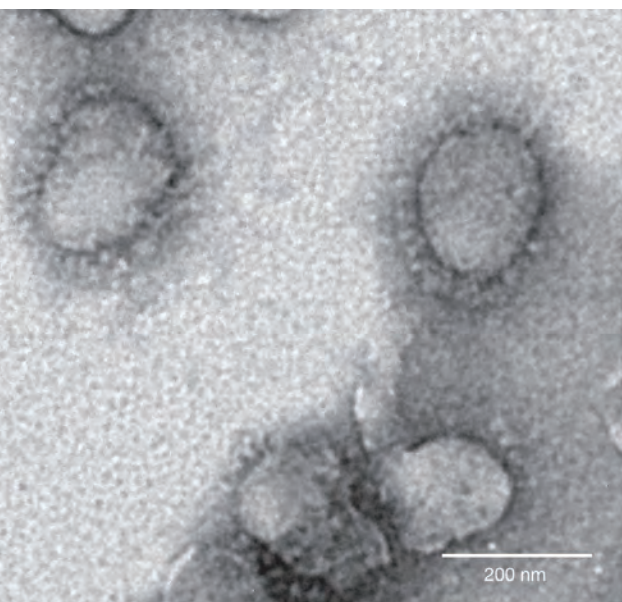


Figure 1. Virions of SARS-like coronavirus isolated from bats. From Ge et al. 2013.

Improving Our Understanding of SARS-like Coronaviruses in Bats

PREDICT isolated for the first time SARS-like coronaviruses from bats sampled in China (Figure 1), fully characterized the whole genome of two novel bat SARS-like coronaviruses, and demonstrated a bat SARS-like coronavirus with 99.98% sequence homology to SARS coronavirus. The virus was found to bind to the human ACE-2 cell receptor, suggesting that direct transmission to humans from bats is possible (Ge et al. 2013). During the 2003 outbreak of SARS in the wet markets of Guangdong province in China, it was thought that bat viruses first infected civets and then evolved to infect people through this intermediate host. However, this study provides compelling evidence that an intermediate host was not necessary. PREDICT has found a surprisingly high number of positives of SARS-like coronaviruses in bats sampled in China. Several of these viruses were novel, and some bats tested positive for multiple novel viruses.

One Health Approach to H7N9 Outbreak Response

The PREDICT team published results demonstrating the first discovery of H9N2 subtype avian influenza virus in wild birds and suggested that these birds may carry H9N2 along migratory routes – highlighting the necessity for continued surveillance of wild birds (Zhu et al. 2013). The PREDICT implementing partners GDCDC and GDIPH organized an expert consulting trip in Guangdong Province during the 2013 H7N9 outbreak, which enhanced surveillance activities, augmented laboratory analysis capacity, and promoted the involvement and coordination of multidisciplinary ministries. PREDICT assisted with the establishment of a Guangdong One Health H7N9 Task Force to coordinate a unified surveillance approach to the H7N9 outbreak among human and animal health sectors. PREDICT facilitated the sharing of expertise and information regarding outbreak response and preparedness between all stakeholders - increasing coordination between human and animal health sectors in China.

CAPACITY BUILDING

One Health Approach to Surveillance and Disease Outbreak Response

PREDICT staff collaborated with scientists and agencies in China to strengthen its public health capacity for identifying new infectious disease threats and early warning disease indicators. PREDICT enhanced surveillance capacity related to pathogen transmission between animals and humans with the goal of incorporating wildlife disease surveillance into standard public health policy in China. Through expanded surveillance and disease modeling, PREDICT

assisted China with developing new tools to target public health resources where they will be most beneficial. After extensive coordinated laboratory analysis with program-engaged partners in China, PREDICT protocols were proven as a valuable viral family level screening tool, as well as a supplemental method to pathogenic-specific real-time PCR. Surveillance activities were coordinated among partners and fostered cross-sectoral collaboration between the human and animal health sectors in China. Information was shared among all partners on a regular basis, which encouraged a One Health approach. Specifically, a hospital-based study and surveillance activities conducted at animal-human interfaces enhanced communication between ministries focused on infectious diseases, which stimulated conversations concerning future inter-ministerial collaborations. These discussions included exchanges regarding efficient strategies for cooperative research and data and sample sharing from both existing and prospective samples and datasets.

PREDICT activities also increased capacity to respond to future disease outbreaks and exerted a positive impact on local and provincial surveillance capacity in Hubei, Yunnan, and Guangdong provinces. In addition, PREDICT China fostered collaboration among provinces and cross-sectoral collaboration between the World Health Organization (WHO), Food and Agriculture Organization (FAO), the Worldwide Scientific Network for Control of Avian Influenza (OFFLU), and the World Organization for Animal Health (OIE).

Training

PREDICT-China trained field and laboratory teams of local scientists in disease outbreak investigation; specimen collection, processing, and preservation; and PREDICT laboratory protocols. Trained personnel followed standardized safety protocols, including use of personal protective equipment, and practiced humane treatment of animals during wildlife sampling activities.

During July 2011, PREDICT staff took part in the Pan Pearl River Triangle Area Emerging Infectious Disease Symposium that was supported by WHO. In August 2012, PREDICT staff attended a ceremony to initiate the modification project of Guangzhou Jiangcun live poultry trade market. This project was supported by both the FAO and China Agriculture Department. In early 2013, the PREDICT China team members participated in the A World United against Infectious Diseases: Cross-sectoral Solutions Conference, which was supported by USAID and WHO, in addition to other organizations and foundations. The PREDICT China team also joined the Sharing Experiences on the Application of One Health Approaches in China meeting, which was organized by WHO and FAO and funded by USAID. To review program success and bring together partners to review the One Health strategy to zoonotic disease control, the PREDICT China team held a forum in Guangzhou on “Surveillance and Epidemiological Research of Zoonotic Diseases & Application of One Health in Disease Control and Prevention in South China”.

SURVEILLANCE

PREDICT primarily conducted zoonotic disease surveillance and research by integrating directly into government laboratories and engaging local CDCs as part of a China public health network. This direct engagement allowed USAID to have the maximum impact and sustainability on local capacity building. PREDICT played a positive role in enhancing capacity building and promoting interprovincial

Swabbing a farmed bamboo rat in Guilin, China.



PHOTO BY HUME FIELD, EHA

collaboration surrounding surveillance of zoonotic disease pathogens and viral/bacterial pathogen identification in China.

Wildlife Disease Surveillance

Wildlife disease surveillance in China focused initially on pathogens commonly detected in bats but was later expanded to include active surveillance for novel pathogens in a variety of wildlife hosts, in particular those taxa that pose a threat to humans or domestic animals. Surveillance activities were conducted in 19 provinces in addition to Beijing, the capital region of China and targeted a variety of high-risk disease transmission interfaces (Figure 2; Table 1). More than half of the samples were collected in the southernmost Provinces of Guangdong, Guangxi, and Yunnan. A total of 3,944 animals were sampled, and most of them were wild-caught bats from a variety of taxonomic families, including Hipposideridae, Vespertilionidae, and Rhinolophidae (Figure 3). Wildlife surveillance efforts were also largely focused on rodents; the majority of which were farm raised bamboo rats (*Rhizomys sinensis*).

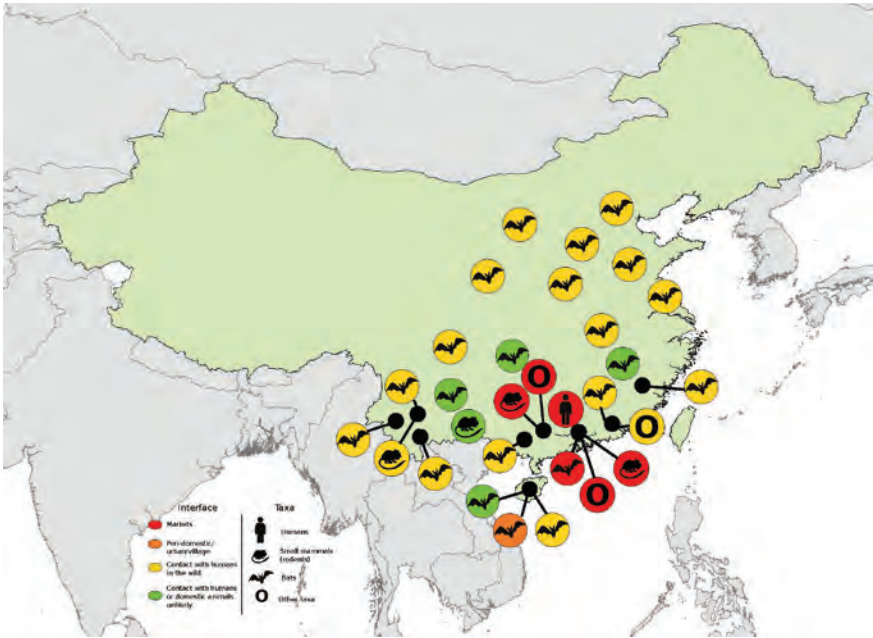


Figure 2. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

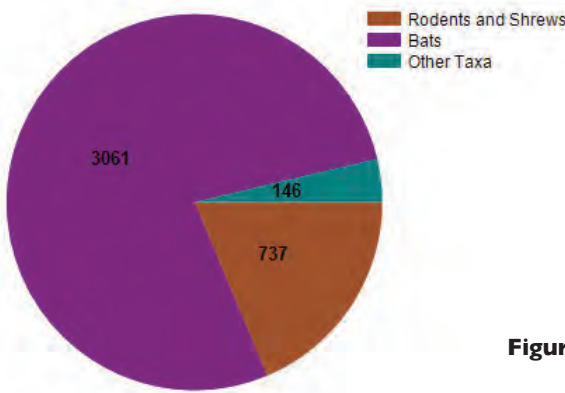


Figure 3. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	144	572	4
Ecotourism and recreational activities	0	1303	4
Extractive industries	0	83	0
In or near human dwellings	7	694	0
Hunted wildlife	0	174	0
Markets	64	0	127
Wildlife preying on livestock or their food	93	0	0
Farmed wildlife	247	0	6
Pristine habitat	182	235	5
Total:	737	3061	146

Human Disease Surveillance

PREDICT-China monitored viral pathogens at high-risk animal-human interfaces and also conducted syndromic disease surveillance. In collaboration with regional Centers for Disease Control and Prevention, PREDICT recruited 1,312 human participants at 12 prefecture-level locations throughout Guangdong Province. Subsequently, serosurveillance was conducted to assess exposure to SARS coronavirus, hantavirus, Ebolavirus, and bunyavirus. Supplemental serological assays were also conducted for leptospirosis, brucellosis, and three types of rickettsial diseases: scrub typhus, spotted fever, and typhus. The PREDICT China team worked jointly with experts at the Yale Occupational and Environmental Medicine Program (YOEMP) to investigate the correlation between specific human practices and infection or transmission of zoonotic disease pathogens. In addition, specimens collected from people with influenza-like illness (ILI) were tested with PREDICT protocols for influenza A and various other viruses associated with ILI symptoms: coronaviruses, paramyxoviruses, hantaviruses, henipaviruses, and arenaviruses.

Additional human disease surveillance investigating acute syndromes, including encephalitis/meningitis, hemorrhagic fever, and fever with thrombocytopenia was conducted through collaborative networks that encompassed prefecture level CDCs and sentinel hospitals. Supported by nine participatory hospitals in Guangdong Province, over 169 samples were collected for this effort. Most samples were collected from prefecture level CDCs and sentinel hospitals within Guangdong Province, although additional samples were obtained from extended regional collaboration with Jiangsu Provincial CDC illustrating the broad interest generated by the PREDICT work in-country.

Disease Outbreak Response and Preparedness

During the H7N9 outbreak, PREDICT facilitated establishment of a Guangdong One Health H7N9 Task Force to improve coordination of surveillance and disease outbreak response efforts among human and animal health sectors in China. PREDICT also provided support to the Influenza Surveillance network in Guangdong Province, which consists of sentinel hospitals and prefecture-level CDCs. Samples were collected from individuals with serious acute respiratory infections and ILI for testing using PREDICT laboratory protocols. Environmental and fecal samples were also collected from wet markets and poultry markets where live birds are sold.

PREDICT team members assisted with testing of wild bird samples obtained from rural areas of Guangdong Province in collaboration with the Ministry of Forestry. Our team's involvement in disease outbreak investigations fostered partnership and trust between PREDICT and key stakeholders in the human and animal health sectors in China and served as a case study of an international One Health response to an emerging disease outbreak in China. PREDICT-China's partners viewed this collaboration as a successful pilot for future international engagement with US partners, particularly during outbreak scenarios.

PREDICT CHINA PUBLICATIONS

PREDICT investigators in China have led and lent support to a number of manuscripts that have been published in peer-reviewed journals. These publications highlight PREDICT's contributions to characterizing important zoonotic viruses circulating in wildlife and people in Asia (see Highlights of PREDICT Publications section for details).

Springborn, M., R.P. Keller, S. Elwood, C.M. Romagosa, C. Zambrana-Torrel, and P. Daszak. **Integrating risk assessment for invasion and disease risk in live animal trade.** (in review)

Jiang, S., C. Ke, M. Akihiko, H.Q. Zhou, K. Zheng, D. Wu, C. Monagin, J.F. He, and J.Y. Lin. **Epidemiology and genetic characterization of dengue fever viruses in Guangdong province, China, 1978-2010.** (in review).

Zheng, X., W. Mai, B. Shu, L. Yi, J. Lu, T. Song, H. Zhong, H. Xiao, D. Guan, J. Wu, L. Liang, C. Monagin, X. Zhang, and C. Ke. **Identification of mild influenza A/H7N9 infection among children in Guangdong Province.** *Pediatric Infectious Disease Journal* (accepted).

Hu, B., A. A. Chmura, J. Li, G. Zhu, G., J. S. Desmond, Y. Zhang, W. Zhang, J. H. Epstein, P. Daszak, and Z. Shi. 2014. **Detection of diverse novel astroviruses from small mammals in China.** *Journal of General Virology*. 95: 2442-2449.

Lu, J., J. Wu, X. Zeng, D. Guan, L. Xou, L. Yi, L. Liang, H. Ni, M. Kang, X. Zhang, H. Zhong, X. He, C. Monagin, J. Lin, and C. Ke. 2014. **Continuing reassortment leads to the genetic diversity of Influenza H7N9 in Guangdong China.** *Journal of Virology* 88(15):8297-8306.

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Zhu, G., Wang, R., Xuan, F., Daszak, P., Anthony, S. J., Zhang, S., et al. 2013.

Characterization of recombinant H9N2 influenza viruses isolated from wild ducks in China. *Veterinary Microbiology* 166(3-4), 327–336. doi:10.1016/j.vetmic.2013.05.013.

Wu, D., H. Zheng, H. Li, C. Monagin, X. Guo, L. Liu, H. Zeng, L. Fang, Y. Mo, H. Zhou, H. Zhang, J. Kou, C. Long, Y. Hiromu, and C. Ke. 2012. **Phylogenetic and molecular characterization of Coxsackievirus A24 variant isolates from a 2010 acute hemorrhagic conjunctivitis outbreak in Guangdong, China.** *Virology Journal* 9:41.

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Recurrent civil war and poverty in DRC have led to population displacements into the forest and consumption of bushmeat as a sole source of protein.

PHOTO BY ANNE RIMOIN (LEFT) AND BY MARCEL OKITOTSHUDI (RIGHT)

DEMOCRATIC REPUBLIC OF CONGO

The Democratic Republic of Congo (DRC), located in the African Great Lakes Region of central Africa, is the second largest country in Africa with 2,345,408 km² of land and 65,710,000 inhabitants (World Bank 2012). The country includes the greater part of the Congo River Basin, which covers an area of almost 100 million hectares. Approximately 45% of the country is covered by primary forest, which is the second largest rain forest in the world after the Amazon. The flora and fauna in the DRC is the most diverse in the African continent (Lambertini 2000).

Numerous outbreaks of zoonotic infections have been reported in DRC, including the first known cases of Ebola virus and human monkeypox in 1976 and 1970, respectively. Since then, the majority of human monkeypox cases have been reported in DRC. Multiple outbreaks of Ebola virus disease have emerged, with up to 318 cases and 280 deaths reported in a single outbreak (WHO 1978). In addition, an outbreak of Marburg virus disease occurred at Watsa in northeastern DRC in 1998 with 154 cases and 128 deaths (Bausch et al. 2006). Additional zoonotic diseases are commonly and regularly reported, including yellow fever, plague, and dengue fever.

A significant percentage of diseases of public health importance in DRC are caused by pathogens originating from wildlife. Bushmeat hunting is common, and this activity has been associated with the emergence of Ebola viral disease. The index case of the first Ebola outbreak in DRC fell ill after consuming fresh and smoked antelope and nonhuman primate meat. In addition, the index case in the 2007 Ebola outbreak was suspected to have been infected from contact with bat

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meat: “the local population described a massive annual fruit bat migration; migrating bats settled in the outbreak area for several weeks and were massively hunted by villagers for consumption” (Leroy et al. 2009). The “putative first case bought freshly killed bats from hunters to eat” (Leroy et al. 2009).

In DRC, significant demographic and anthropogenic changes have occurred since the 1980s. These changes have likely increased contact between local populations and wildlife, especially in and around forested areas. Indeed, recurrent civil war and poverty have led to population displacements into the forest and consumption of bushmeat as a sole source of protein. In addition, forest clearing for farming, extractive industries, lodging, and firewood collection has favored increased contact with nonhuman primates, rodents, bats, and other wildlife species known to harbor zoonotic pathogens.

There was a critical need in DRC to establish a well-implemented surveillance system at the human-wildlife interface and to adopt a One Health approach to prevention and control of emerging and re-emerging infectious diseases. In response, PREDICT collaborated with the Government of DRC to create wildlife surveillance expertise and develop diagnostic laboratory and disease outbreak response capacities. Through these activities, PREDICT evaluated the diversity of wildlife viruses with potential for human infection and explored the implications of human and wildlife interactions on disease transmission and emergence in order to identify strategies for minimizing the risk of cross-species transmission.

In DRC, the PREDICT project built on the experience of human monkeypox and other active zoonotic surveillance programs that were initiated in concert with the Institut National De Recherche Biomedicale (INRB; English translation: National Institute of Biomedical Research) and the Kinshasa School of Public Health (KSPH) in 2000. These two institutions were the first in-country partners of PREDICT. The INRB, which is DRC’s national public health laboratory, provided space for a new laboratory to perform molecular tests on wildlife samples and a room for the project office, as well as scientific and administrative expertise. The director of INRB was the primary project point of contact at the DRC Ministry of Health. The KSPH facilitated administrative clearances with the DRC Government for the official recognition of the project and provided staff for the project. The Director of KSPH was the point of contact for the project at the Ministry of Higher Education and Scientific Research. PREDICT also collaborated with the Ministry of Health National Program against monkeypox and viral hemorrhagic fevers and the Central Direction for disease surveillance.

PARTNERS

PREDICT partners in DRC included Metabiota, Institut National de Recherche Biomedicale (INRB), Kinshasa School of Public Health, Ministry of Environment – Institut Congolais pour la Conservation de la Nature (ICCN), the Ministry of Health – Direction of Disease Surveillance, and USAID.

Other in-country partners included:

- Congolais pour la Conservation de la Nature (ICCN)
- Central Veterinary Laboratory of Kinshasa
- University of Lubumbashi

- University of Kisangani
- Lola Ya Bonobo
- Mona Paradis
- Societe des Jesuites
- Botanical garden of Kisantu
- Mountain Gorilla Veterinary Project

EPT partnerships included:

The PREDICT project conducted several activities in partnership with RESPOND, WHO, and the US CDC, including implementation of One Health activities by organizing meetings with key ministries; preparation of a curriculum for health, veterinary, and nurses training at high school and college levels; training of local staff in the Equateur province in investigation activities for human monkeypox; and responding to outbreaks in bonobos, chikungunya in Kinshasa, and during the 2012 and 2014 Ebola outbreaks.

WWF, through its “CARPE” and “PICBOU” projects, partnered with PREDICT by jointly visiting hotspot regions in the country, especially in the area of the Salonga National Park where the WWF and PREDICT staff worked together to sensitize local community members in the protection of nature and prevention of zoonotic infections by safe handling of bushmeat, avoiding risky behaviors, such as transporting and butchering found dead animals, and reporting abnormal die-offs among wildlife.

PREDICT regional collaborative laboratories:

The PREDICT project in DRC worked in partnership with the Centre International de Recherche Medicale de Franceville (CIRMF) in Gabon, the Center for Infection and Immunity (CII) at Columbia University in New York, USA, and the University of California, San Francisco.

PREDICT also collaborated with the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) monkeypox project in Kole.

A regional collaboration was established between PREDICT staff within the central African countries: DRC, Cameroon, Gabon, and Republic of Congo. This collaboration focused on training and sharing challenges and solutions, providing for “cross-pollination” and sustainability of One Health activities regionally.

The PREDICT team in Isiro coordinating with the WHO office.



PHOTO BY GUY MIDINGI

MAJOR ACHIEVEMENTS:

- Assisted with the response to a variety of disease outbreaks in DRC, including Ebola virus disease. As a result of the success of PREDICT's assistance during the outbreaks, the PREDICT laboratory at the National Institute of Biomedical Research was officially integrated into the viral hemorrhagic fever national surveillance system (see Success Stories for more information). PREDICT laboratory staff were included as members of the scientific and laboratory committee of the National Commission for Ebola Outbreak Response.
- The PREDICT laboratory diagnosed the first human samples from suspected cases of viral hemorrhagic fever (VHF) from Boende in the Equator province of DRC. The samples tested positive for Ebola Zaire using PREDICT protocols followed by sequencing. These results allowed the DRC Government to officially declare the 2014 Ebola Outbreak and to set up an outbreak response plan. The Ebola virus sequences were publically released via Genbank.
- Enabled discovery of a new, potentially highly-pathogenic human virus, the Bas-Congo virus (BASV) that caused an outbreak of viral hemorrhagic fever in the Mangala village and surrounding area in Bas-Congo Province (see Success Stories for more information).
- Engaged local communities by organizing regular village meetings during which community members were informed of surveillance activities, briefed on safe wildlife hunting and butchering techniques, and trained in the collection of samples from hunted wild animals (see Success Stories for more information).
- Developed infrastructure for and implemented a wildlife surveillance program. Equipment was procured and tested for utilization as a deployable laboratory that can be quickly set up in the field for outbreak response, including a real-time PCR machine, satellite phone, and a broadband satellite terminal to allow transmission of data from anywhere in the field – this equipment was deployed during the 2014 Ebola virus outbreak.
- Augmented diagnostic capacity through infrastructure improvements, including installation of a liquid nitrogen plant, which provided continuous cold-chain for sample collection throughout the country. PREDICT staff also optimized and implemented testing for 21 virus families.
- Sampled a total of 3,459 wild animals including bats, nonhuman primates, rodents and shrews, and animals from other taxa.

PREDICT staff removing bats from the net for sample collection.



PHOTO BY PRIME MULEMBAKANI



Village meeting: sensitization on healthy hunting and butchering, by the PREDICT field coordinator.

- Assessed genomic diversity of the Monkeypox virus among people with primary and secondary cases of infection in DRC from 2005-2007. The research team detected four distinct lineages of the virus and discovered a deletion that resulted in gene loss that appears to correlate with human-to-human transmission and disease severity.
- Detected a novel Simian Foamy Virus in healthy women from DRC. The Simian Foamy Virus (SFV) originated from the Angolan colobus (red-tailed monkey).

SUCCESS STORIES

Early detection of an Ebolavirus outbreak in the Democratic Republic of Congo

PREDICT assisted in the early detection of an outbreak of Ebolavirus in DRC in August, 2014. On August 11, a woman died in the village of Ikanamongo in Equateur Province of an unknown cause with symptoms of a hemorrhagic fever, and by August 18 reports of 24 suspected cases in nearby villages were received in the capital city of Kinshasa. A national response team went to the remote site and was able to deliver samples from eight individuals, who were in direct contact with the index case, to Kinshasa by August 22. However, key national virology staff were in West Africa at the time responding to governmental requests to assist in the Ebolavirus outbreak in that region. Consequently, the deputy director of INRB requested that the PREDICT laboratory, hosted at INRB, conduct the diagnostic testing. Ebolavirus was confirmed in samples from the outbreak using PREDICT protocols on August 23. Based on the early results of the PREDICT/INRB laboratory tests, the DRC government was able to enact disease control measures such as quarantine, dispatch of a mobile laboratory, and contract tracing, among other measures. Consequently, the prompt assistance of the PREDICT/INRB laboratory may have facilitated an early response and increased the likelihood of successful containment procedures.

Community Engagement

PREDICT staff worked closely with local community members to raise awareness on the risk of zoonotic infections, especially among hunting communities in forested areas. Community members participated in the surveillance activities by collecting bushmeat samples from their hunted wild animals. Participants received training on sample collection and were informed of the zoonotic pathogen risk associated with wildlife hunting and consumption. More than 2,000 bushmeat samples collected by community members were received at the laboratory in Kinshasa. The laboratory diagnostic results and implications to human health were shared at village meetings in order to increase local knowledge regarding zoonotic pathogens and the risk of spill-over to people through hunting, butchering, and consumption of bushmeat.

Integration of PREDICT into the Viral Hemorrhagic Fever National Surveillance System

PREDICT assisted with the response to a variety of disease outbreaks in DRC, including Ebola in 2012 and 2014, encephalomyocarditis virus in bonobos, *Brucella abortus* in cows from the Masisi District, and Chikungunya in Kinshasa. Due to the success and visibility of PREDICT's assistance during these outbreaks, the PREDICT laboratory at the INRB was officially integrated into the national surveillance system for VHF. The laboratory received samples from VHF suspect cases for screening using PREDICT diagnostic protocols. Likewise, samples from people with fevers of unknown origin were provided to the PREDICT laboratory for pathogen detection and discovery.

Discovery of a Novel Human Pathogen Associated with Acute Hemorrhagic Fever in People

In collaboration with the Ministry of Health, the PREDICT DRC team, supported by the advanced diagnostic capability of the PREDICT Gabon team, obtained samples from survivors of a VHF outbreak in Mangala village, Bas-Congo Province. PREDICT used deep sequencing, a method for generating millions of DNA sequence reads from clinical samples, to discover a novel rhabdovirus (Bas-Congo virus, or BASV) associated with this outbreak of human cases of acute hemorrhagic fever in DRC.

Bas-Congo virus was detected in the blood of the lone survivor. The genome of BASV, assembled using a technique that was optimized through a larger PREDICT-funded viral discovery project at the University of California, San Francisco, revealed that the virus was very different from known rhabdoviruses (Grard et al. 2012). Antibodies to BASV were detected in one health care worker and the lone survivor. Although the source of the virus remains unclear, findings suggest that BASV is an emerging pathogen associated with acute hemorrhagic fever in Africa and may be spread by human-to-human contact. The discovery of BASV in Central Africa suggests that additional rhabdoviruses of clinical and public health importance await discovery, especially in these poorly investigated geographic regions. Active surveillance is needed to identify the source of infection and fully ascertain the public health significance of BASV infection.

CAPACITY BUILDING

Training

Surveillance and Disease Outbreak Response. PREDICT-DRC provided a variety of training sessions for project staff and collaborators to improve capacity for disease surveillance and outbreak response. PREDICT DRC staff received training on a broad range of topics, including but not limited to: wildlife capture and handling, specimen collection and processing, cold chain management, laboratory diagnostics and safety, biosafety and PPE use, and database management. Training on proper use of PPE was also provided to staff and participants from ministries and other collaborating organizations. Specific fit testing, using internationally standardized procedures, was conducted on individual staff members to ensure the appropriate PPE size and fit. In addition, two PREDICT staff and one scientist from the INRB were trained on biorisk management and shipping of Class 6.2 dangerous goods and dry ice, by the Sandia National Laboratories in the USA. These individuals are now equipped to train other in-country laboratory workers on these topics.

Village meeting: the PREDICT field veterinary technician and DRC MoH director of monkeypox explain to community members how to collect bushmeat samples from hunted animals.



PHOTOS BY PRIME MULEMBAKANI



The PREDICT field laboratory in Kole during a training session of local staff.

PREDICT also provided unique training opportunities to staff and officials from collaborating ministries, NGOs, and the national veterinary laboratory. Participants included both senior officials responsible for management and policy development and regional staff responsible for day-to-day management of wildlife conservation and veterinary services. Staff from the Ministry of Environment and Livestock were trained on wildlife capture and handling, wildlife sampling, specimen storage and transport, proper PPE use, and risk communication regarding suspected zoonotic disease cases and anomalous animal health events (i.e. disease outbreaks and unexpected mortality events). In addition, senior staff from the Ministry of Environment were trained on the

use of the PREDICT assessment tool to assess new hotspot sites and new human-wildlife interfaces to be targeted for surveillance activities. Local veterinary technicians were trained on biosafety during animal disease outbreaks. Furthermore, the director of the National Program for Monkeypox and Viral Hemorrhagic Fevers received training on electrocardiography at the Kinshasa Provincial General Hospital, which provided a point-of-care diagnostic modality for assessing the impacts of human monkeypox and viral hemorrhagic fever infections on cardiac function of individuals during outbreaks.

Train-the-trainer program. To ensure sustainability of the enhanced surveillance capacity in DRC, PREDICT staff organized a train-the-trainer program: local staff in rural forested areas were trained as trainers of community members to expand the understanding of the origin of zoonotic diseases and measures to reduce the risk of infection and to provide opportunities to participate in disease surveillance efforts by providing bushmeat samples from hunted and butchered animals. Many villages in the Kasai-Oriental province participated in this program and provided bushmeat samples for testing. PREDICT staff organized regular village meetings during which community members

were informed of surveillance activities, sensitized to biosafety measures that can be taken to reduce exposure to zoonotic pathogens during hunting and butchering activities, and trained in the collection of bushmeat samples from hunted wild animals. Several communities participated in the surveillance efforts by providing high quality samples from hunted nonhuman primates, rodents, and other wild animal species. In addition, communities were provided information on how to report suspected cases of monkeypox and VHF.

Laboratory Improvements

PREDICT provided important equipment to support surveillance activities, including PCR machines and other supplies to conduct laboratory analyses, and multiple freezers to improve storage capacity for biological samples. In addition, PREDICT installed a liquid nitrogen plant to provide continuous cold-chain for sample collection throughout the country. The laboratory was equipped to perform rapid serologic tests, DNA and RNA extractions, and conventional PCR assays.

PREDICT staff optimized and implemented testing of a full virus panel composed of 21 virus genera/families: arenaviruses, coronaviruses, filoviruses, flaviviruses, enteroviruses, paramyxoviruses, rhabdoviruses, lyssaviruses, hantaviruses, alphaviruses, henipaviruses, bunyaviruses, retroviruses, influenza viruses, simian foamy viruses, herpes viruses, bocaviruses, poxviruses, adenoviruses, and polyomaviruses. The DRC laboratory staff also optimized a PCR amplification method for detection of specific viruses, including hepatitis B virus, Simian T-Cell Leukemia virus, encephalomyocarditis virus, and the newly discovered Bas-Congo virus.

Equipment was procured and tested for its utility as a deployable laboratory that can be rapidly applied for field use during an outbreak response. The equipment included a real-time PCR machine and technology to transmit data and communications from anywhere in the field, which allows for real-time reporting of results.

SURVEILLANCE

At the launch of the PREDICT project in DRC, the team conducted, in collaboration with DRC Government institutions, NGOs, and other partners, an assessment of current data and surveillance activities at the human-wildlife interface. This informed the selection of surveillance sites where high-risk wildlife, specifically bats, rodents, and nonhuman primates, have a high likelihood of contact with humans.

The PREDICT DRC field team coordinated with partners including government, NGOs, and communities to prioritize surveillance sites and conduct field efforts in eight out of the 11 provinces of the country. Samples were collected from hunted animals, nonhuman primates at sanctuaries (sick individuals and recently accessioned primates from the wildlife trade), rodents and bats in and around human dwellings and in areas with ecotourism, and wildlife surrounding disease outbreaks (Figure 1; Table 1). The PREDICT team sampled a total of 3,459 wild animals (Figure 2), and collected a total of 21 samples from humans with suspected VHF, two of which tested positive for Ebola Zaire virus.

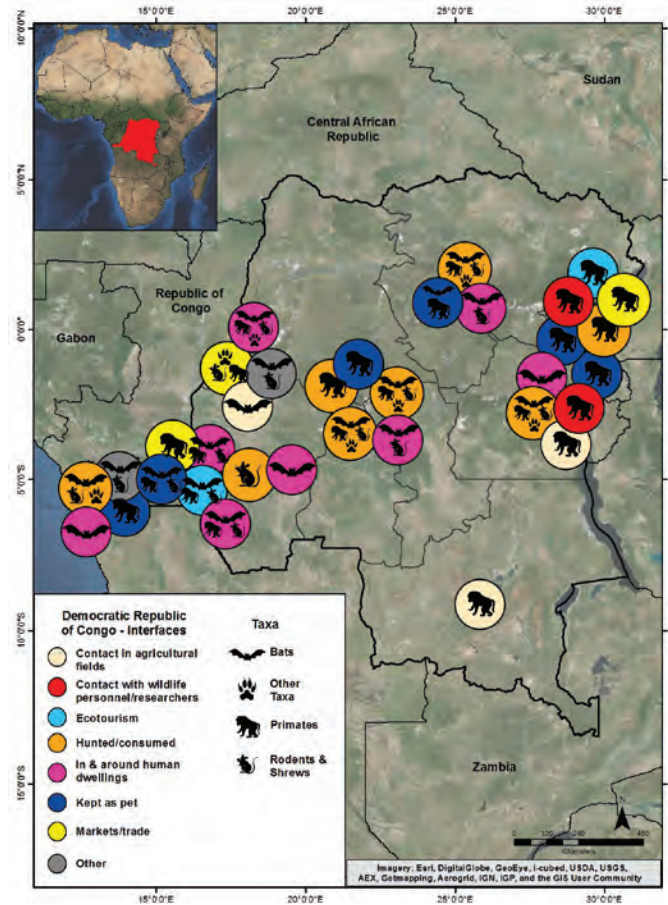


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

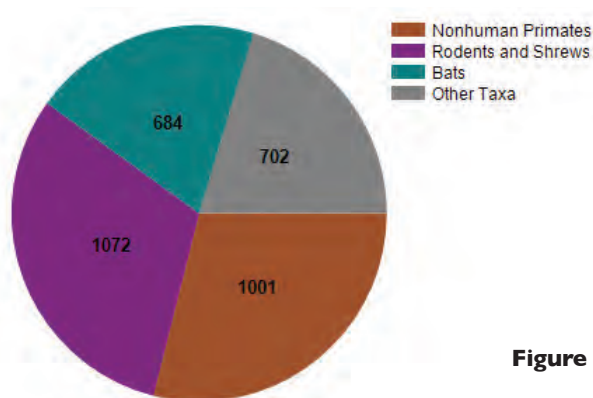


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	1	0	14	0
Ecotourism and recreational activities	24	18	78	0
In or near human dwellings	16	49	397	3
Hunted wildlife	679	935	75	687
Wildlife trade	1	0	0	0
Markets	46	1	6	12
Zoos and sanctuaries	217	7	64	0
Protected areas	17	0	0	0
Other high-risk interfaces	0	62	50	0
Total:	1001	1072	684	702

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

The PREDICT staff provided expertise and assistance during five (three human and two animal) disease outbreaks

- **Ebola outbreak in Boende, 2014.** The PREDICT team was responsible for the initial diagnosis of Ebolavirus as the cause of the outbreak. The team confirmed that the outbreak in Boende was caused by the same species of Ebolavirus as the West Africa outbreak, but that it was not closely related, suggesting that the DRC outbreak was an independent spillover event. Based on the early results of the PREDICT laboratory analysis, the DRC government was able to enact disease control measures such as quarantine, dispatch of a mobile laboratory, and contract tracing, among other measures. Consequently, the early assistance of the PREDICT laboratory may have facilitated an early response and increased the likelihood of successful containment procedures. At the time of publication of this report, PREDICT continued to play a role in the outbreak response efforts (See Success Stories above).
- **Ebola outbreak in Isiro, 2012.** During the outbreak, the PREDICT team participated in the outbreak response with other national and international organizations. Samples were collected from nearby bats and rodents, as well as from nonhuman primates kept as pets. In addition, samples collected from pigs were also received for testing following rumors of a swine die-off. Local personnel from the Ministries of Environment and Livestock were trained in PREDICT field protocols, including wild animal sampling, sample shipment, personal protection, safe handling of animal carcasses, and reporting of animal health events. PREDICT staff was involved in regular government meetings held during the outbreak and provided expertise on implementation of a One Health approach to the response.
- ***Brucella abortus* outbreak in cows in the Masisi District, Eastern DRC.** In September–October 2010, a farmer with a herd of 1,000 cows experienced an outbreak of neonatal calf mortality. The PREDICT staff assisted the Central Veterinary Laboratory in Kinshasa with the response by coordinating sample shipment and testing. The Central Veterinary Laboratory isolated *Brucella abortus*.



Caring for a sick bonobo at the sanctuary.

- **Chikungunya outbreak in humans and bonobos at the bonobo sanctuary in Kinshasa.** In January 2012, the PREDICT staff was called to the sanctuary Lola Ya Bonobo to investigate cases of illness in newly accessioned bonobos and sanctuary staff. After organizing a meeting with the director of INRB and Ministry of Health (MoH) staff, serological tests were performed on samples collected from the bonobos and staff. A diagnosis of chikungunya virus was made. The MoH and contingency were alerted of the results and control measures were implemented. The PREDICT team mobilized a team of entomologists who trapped mosquitos at the sanctuary for screening. Information was shared with the MoH official in charge of disease surveillance. All health facilities in Kinshasa were also made aware of the outbreak.

- **Encephalomyocarditis outbreak among bonobos at the bonobo sanctuary in Kinshasa.** From January through May 2012, four bonobos died at the sanctuary. PREDICT took the lead in coordinating the investigation and response to this outbreak. We arranged a veterinary visit to the site for a clinical investigation and shipped samples to CIRMF and the Center for Infection and Immunity (CII) at Columbia University, where they were screened for a wide range of pathogens. Specimens tested positive using molecular and histological assays for the encephalomyocarditis virus (EMCV). Control measures were implemented in and around the sanctuary to minimize the risk of infection.
- **Outbreak of viral hemorrhagic fever in Bas-Congo.** In May-June 2009, three suspect cases of VHF presented in the Bas-Congo province in western DRC. The first two cases died and the third one survived. All samples were negative for known viral hemorrhagic fever pathogens. PREDICT conducted an investigation in the area and collected samples from the lone survivor and his close contacts. These efforts led to the discovery of a novel virus, belonging to the rhabdovirus family, named Bas-Congo virus (BASV). PREDICT also collected wildlife samples in the immediate area of the outbreak to investigate potential reservoir species.

VIRUS DETECTION AND CHARACTERIZATION

PREDICT made it possible to discover a potentially highly-pathogenic human virus, the Bas-Congo virus (BASV), a novel rhabdovirus, that caused an outbreak of viral hemorrhagic fever in the Mangala village and surrounding area, Bas-Congo Province, via deep sequencing in collaboration with the University of California, San Francisco (Grard et al. 2012).

In addition, PREDICT researchers assessed the genomic diversity of the Monkeypox virus in samples obtained from humans with primary and secondary cases of infection from 2005-2007 (Kugelman et al. 2014). The results revealed four distinct lineages and a deletion that resulted in gene loss that appears to correlate with human-to-human transmission and disease severity. The results suggest frequent spillover of the viruses from animals to people and potential increased adaptation to humans (Kugelman et al. 2014).

Further, PREDICT researchers screened a large population of rural DRC inhabitants for simian foamy virus infection and investigated risk factors for exposure (Switzer et al. 2012). Sequence analysis revealed human infection with new simian foamy viruses from Angolan colobus and

red tailed monkeys, two commonly hunted nonhuman primate species. Unlike previous studies, women were not at lower risk of infection with simian foamy viruses relative to men (Switzer et al. 2012). Men commonly hunt while women are responsible for food preparation, suggesting that exposure may be as or more frequent with bushmeat preparation compared to hunting.

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In Gabon, hunting of wildlife is an important cultural and economic activity, especially in rural communities where bushmeat serves as a substantial source of dietary protein and income.

PHOTOS BY MATTHEW LEBRETON

GABON

The Gabonese Republic is located on the Gulf of Guinea in Central Africa, crossed by the equator and bordered by the Republic of Congo, Cameroon, and Equatorial Guinea. This country occupies 270,000 km² of land, three-fourths of which is covered by tropical forest. Thirteen national parks have been created in Gabon, representing 10% of the territory, which includes the Lopé National Park, a UNESCO World Heritage site. In the western and southeastern regions of the country, coastal lowlands and savannah landscapes are the predominant ecosystems, respectively. Watersheds of the Ogooué River occupy a significant part of the country (Encyclopedia Britannica 2014). The flora and fauna in Gabon spans a remarkable biodiversity, undoubtedly one of the most diverse in the world. About 200 different mammalian species are found in Gabon, including rare species such as pangolins and sun-tailed monkeys. In addition, the country's forests are an important refuge for gorillas and chimpanzees.

According to the United Nations, Gabon is a prosperous country with the highest human development index in the African continent. Although intensive agriculture and ranching are poorly developed, there are significant forest resources. However, significant anthropogenic disturbance has occurred in the forests due to exploitation of oil, minerals, and hydropower. Despite the official figures, a significant proportion of the population remains poor in Gabon. The population is estimated to be over 1,500,000, consisting of more than 40 ethnic groups. The country has a very low population density (six inhabitants per km², as compared to 37 per km² for the African continent as a whole; Encyclopedia Britannica 2014). However, half of the population lives in the two major cities: Libreville, the capital in north-west and Port-Gentil, the economic capital and main harbor in the east, positioning Gabon as one of the most urbanized countries in Africa.

FOR A SUMMARY OF THIS SECTION GO TO WWW.GABON.PREDICT.GLOBAL

In 1979, the Centre International de Recherches Médicales de Franceville (CIRMF) was created in Gabon and has served as a modern research facility for Central Africa. CIRMF focused its activities in the early 1980s on HIV/AIDS and malaria; the two major pandemics affecting Africa. In the late 1990s, emerging infectious disease threats, such as Ebola virus disease, Chikungunya fever, and influenza A (H1N1) led CIRMF to adopt the One Health concept. Moving forward, CIRMF conducted scientific research programs that focused on infectious human pathogens and their zoonotic origins.

Hunting of wildlife is an important cultural and economic activity in Gabon. Bushmeat is a major source of dietary protein and income for rural communities. However, hunting carries substantial risk for inter-species transmission of zoonotic pathogens and is assumed to be the mechanism by which HIV and other dangerous zoonotic pathogens first spilled over into humans (Pike et al. 2010). Several pathogens have emerged in the forests of Gabon, including viruses responsible for hemorrhagic fever outbreaks. Human and animal outbreaks of Ebola have occurred in recent decades, and Marburg virus has been isolated from fruit bats in Gabon.

PREDICT partnered with CIMRF to enhance the country's wildlife surveillance, diagnostic laboratory, and outbreak response capacities. PREDICT-Gabon focused on detection of zoonotic pathogens of wildlife origin in areas where anthropogenic change and human behaviors have resulted in increased contact between wildlife and human populations, and therefore a heightened risk of inter-species transmission.

PARTNERS

PREDICT partners in Gabon included:

- Centre International de Recherches Médicales de Franceville (CIRMF)
- Metabiota
- Agence Nationale des Parcs Nationaux (ANPN)
- Conseil Africain et Malgache pour l'Enseignement Supérieur (CAMES)
- Centre National de la Recherche Scientifique (CENAREST)
- Ecole Doctorale Régionale d'Afrique Centrale (EDR)
- Food and Agriculture Organization (FAO)
- Institut National Supérieur d'Agronomie et de Biotechnologie (INSAB)
- Institut Pasteur (IP)
- Institut pour la Recherche et le Développement (IRD)
- Laboratoire National de Santé Publique (LNSP)

Egyptian Fruit Bats, *Rousettus aegypticus*, during sampling by PREDICT staff in cave at Belinga, Gabon.



PHOTO BY MATTHEW LEBRETON AND BRAD SCHNEIDER

- French Ministry of Foreign Affairs (MAE)
- USAID
- Gabonese Ministry of Education and Research (MoER)
- Gabonese Ministry of Health (MoH)
- Gabonese Ministry of Water and Forest (MoWF)
- Projet Grands Singes (PGS)
- Université des Sciences de la Santé - Omar Bongo (USS-OB)
- Université des Sciences et Techniques de Masuku (USTM)
- Wildlife Conservation Society (WCS)
- World Health Organization (WHO)

MAJOR ACHIEVEMENTS

- Served as a regional diagnostic laboratory for virus detection and characterization and made significant advancements in our understanding of highly pathogenic viruses emerging in Central Africa (see Success Stories for more information).
- Characterized the Zaire ebolaviruses from the 2007 and 2008 outbreaks in Luebo, Democratic Republic of Congo (DRC; see Success Stories for more information).
- Confirmed results produced by the Institut National de Recherche Biomedical (INRB) in DRC that the 2014 Ebola outbreak was distinct from the West Africa Ebola virus outbreak, and completed full genome sequencing of the virus.



The PREDICT field team collects specimens from bats captured at the Belinga caves near Makokou.

- Enhanced disease surveillance capacity and implemented a surveillance network that incorporates both the animal and human health sectors, collecting specimens from 3,491 animals.
- Established a national surveillance network for specific syndromes including hemorrhagic, febrile, enteric, and flu-like illnesses in association with the high Gabonese authorities within the nine Gabonese provinces; a reference physician was identified in each regional hospital to facilitate sample collection.
- Developed an animal disease reporting system that meets international standards in partnership with the Centre National de la Recherche Scientifique, the Ministry of Health, and the Agence Nationale des Parcs Nationaux.
- Expanded implementation of advanced molecular laboratory diagnostics to detect and characterize both known and novel zoonotic viruses in wildlife and optimized and implemented 21 viral family testing protocols.

SUCCESS STORY

Regional Diagnostic Laboratory for Human and Wildlife Disease Events

As of 2014, the PREDICT in-country partner laboratory, CIRMF, had increasingly become the reference diagnostic testing facility for Central Africa. In particular, specimens from unusual and/or highly pathogenic human and wildlife disease cases were routinely forwarded to CIRMF for assistance in detection and characterization of the etiologic agent.

During PREDICT, CIRMF collaborated on a number of investigations during suspected VHF outbreaks within the Central African region. For example, PREDICT and its partners reconstructed the genome of a novel rhabdovirus (Bas-Congo virus, or BASV) associated with three human cases of acute hemorrhagic fever in DRC in 2009 (Grard et al. 2012). The rhabdovirus family had never been documented to cause hemorrhagic fever in people.

Given the severity of the associated syndrome, this virus is potentially a new BSL-4 virus, representing a credible threat to public health. Identification of this virus expands the panel of viruses in diagnostic tests when viral hemorrhagic fever is suspected, improving our ability to identify etiological agents in similar outbreaks.

In addition, PREDICT characterized the Zaire ebolaviruses from the 2007 and 2008 outbreaks in Luebo, DRC (Grard et al. 2011a). The two Luebo ebolaviruses were found to be distinct from previous ebolaviruses characterized in DRC and in the Gabon-Republic of the Congo area, suggesting that many Ebola virus outbreaks do not result from viral spread from previously identified foci. Instead, the outbreaks likely arise from independent emergence events, and local wildlife populations (most likely bats) become infected and allow viral persistence and reemergence from year to year (Grard et al. 2011).

Furthermore, CIRMF used phylogenetic analysis to investigate an isolated human case of Crimean-Congo hemorrhagic fever (CCHF) in DRC in 2008. The analysis suggested that CCHF virus circulation in Central Africa has been occurring for a long time, despite the absence of reported human cases, and that many human infections have likely been overlooked. The sporadically reported human cases are associated with a sylvatic cycle in Central Africa where deforestation may heighten the risk of re-emergence (Grard et al. 2011b).

CAPACITY BUILDING

Laboratory Diagnostic Capacity

During the PREDICT project, CIRMF diagnostic infrastructure was substantially enhanced through installation of new laboratory equipment that allowed for rapid automation and high-throughput techniques for pathogen discovery and characterization. PREDICT-Gabon optimized and implemented protocols to detect both known and novel viruses in 22 viral families/genera of high-consequence (alphaviruses, arenaviruses, astroviruses, bunyaviruses, coronaviruses, enteroviruses, hantaviruses, henipaviruses, filoviruses, flaviviruses, lyssaviruses, orbiviruses, paramyxoviruses, phleboviruses, rhabdoviruses, influenza viruses, retroviruses, adenoviruses, bocaviruses, herpes viruses, poxviruses, and polyomaviruses). These protocols were used to screen clinical specimens from both wildlife and humans. Testing was facilitated through development of universal control materials allowing test validation without cultivation or shipment of viruses, or maintenance of unstable RNA virus control material.

Training

A series of trainings were provided for personnel, including wildlife capture and sampling, bushmeat sampling, specimen handling and shipment, PPE use and biosafety, laboratory methodology, laboratory safety, and data management. One member of the PREDICT Gabon team received regional training from the Cameroon team in order to optimize the PREDICT protocols in Gabon, refine wildlife collection techniques, share laboratory experiences, and build a regionally sustainable collaboration among PREDICT teams. This training was applied and was conducted within the laboratory and during field sampling trips with the Cameroon team. The PPE training was extended to participants from ministries and other collaborating organizations. As part of this training, specific fit testing using internationally standardized procedures was conducted on individual

staff members to ensure the appropriate PPE fit. PREDICT laboratory staff was comprehensively trained in all steps of sample processing and pathogen discovery techniques. Molecular biology expertise was improved locally and used for the duration of the project. In addition, PREDICT staff contributed to the training of graduate students, healthcare workers, and local scientists on a range of topics, including specimen collection, transport, processing, and laboratory analysis.

SURVEILLANCE

PREDICT enhanced surveillance capacity in Gabon by augmenting resources to conduct active and opportunistic sampling of high priority wildlife taxa. Samples were collected throughout the country from bats, rodents, nonhuman primates, and ungulates at high-risk disease transmission interfaces between wildlife and humans (Figure 1 and Table 1). In addition, bushmeat samples were collected from hunters and markets. During the PREDICT project, samples were collected from 3,491 animals (Figure 2; bats, rodents, nonhuman primates, and ungulates). A strategic sample selection system was utilized to prioritize testing based on taxa, specimen type, and risk interface targeted to maximize discovery of viruses of public health significance.

PREDICT staff preparing equipment for sampling bats at Belinga, Gabon.



PHOTO BY MATTHEW LEBRETON

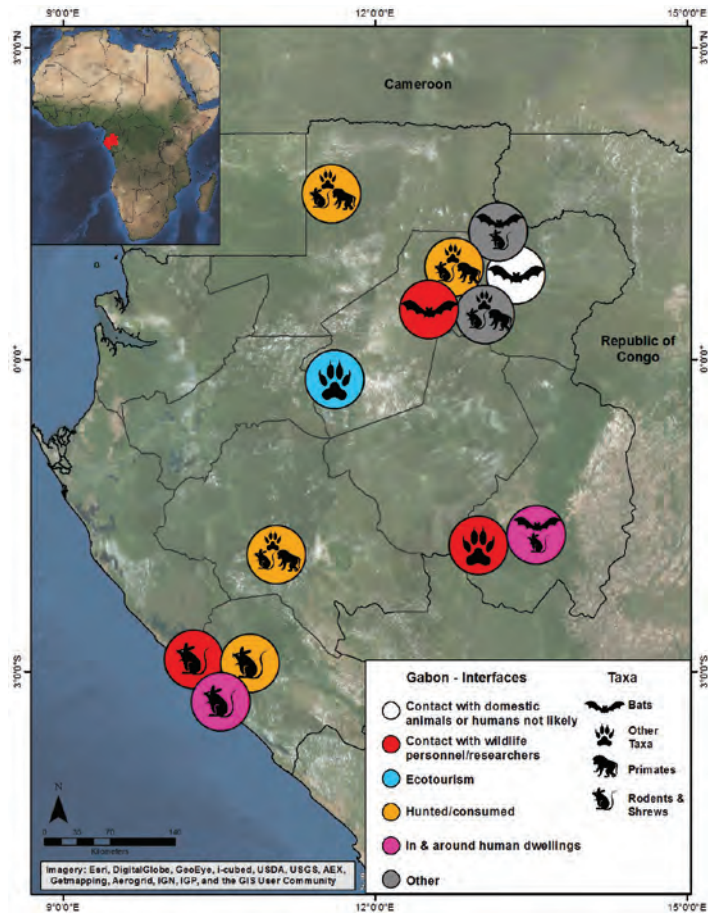


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

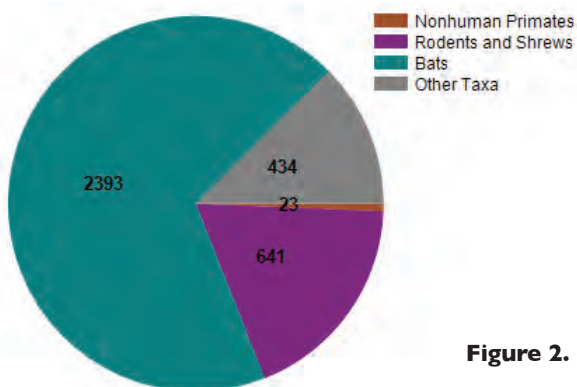


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Ecotourism and recreational activities	0	0	0	340
In or near human dwellings	0	322	75	0
Hunted wildlife	20	21	200	45
Pristine habitat	0	0	15	0
Protected areas	0	140	1	30
Other high-risk interfaces	3	158	2102	19
Total:	23	641	2393	434



PHOTOS BY PIERRE BECQUART AND ERIC LEROY

PREDICT conducted a field mission in a remote Gabonese village, during a national serosurveillance effort, to provide communities with education on infectious disease risks.

In addition to wildlife disease surveillance, a national syndromic surveillance system was established in association with the high Gabonese authorities within the nine Gabonese provinces; a reference physician was identified in each regional hospital to facilitate sample collection and shipment to CIRMF. Hemorrhagic, febrile, enteric, and flu-like syndromes were targeted.

Furthermore, PREDICT-Gabon contributed to an animal disease reporting system for national authorities responsible for animal disease management, including the Centre National de la Recherche Scientifique, the Ministry of Health, and the Agence Nationale des Parcs Nationaux. This was the first coordinated mechanism for animal disease reporting in the country.

PREDICT expanded the implementation of advanced molecular laboratory diagnostics to detect and characterize known and novel viruses. CIRMF developed national partnerships with ministries, universities, and other public health facilities throughout the country.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT and its partner CIRMF assisted in the diagnosis of unusual human and wildlife diseases regionally. Outbreak response was coordinated in partnership with the Gabonese ministries. Multidisciplinary field teams from CIRMF collected specimens during wildlife and/or human disease outbreaks and deployed a mobile laboratory with biosafety equipment during suspected outbreaks within the region. Furthermore, CIRMF benefited from a BSL-4 rated high containment laboratory and now has the capacity to employ next generation sequencing technology during outbreak investigations.

From 2009 to 2012, wildlife and human samples collected during acute hemorrhagic fever outbreaks in the DRC were routed to CIRMF for analysis. Samples were tested for Zaire ebolavirus, Sudan ebolavirus, Ivory Coast ebolavirus, Bundibugyo ebolavirus, Marburg virus, Lassa virus, CCHF virus, Rift Valley fever virus, yellow fever virus, dengue virus, and Chikungunya virus. PREDICT protocols were also used to test for novel viruses. In 2010, a novel rhabdovirus (Bas-Congo virus, or BASV) was discovered during a VHF outbreak (Grard et al. 2012), suggesting the emergence of a new pathogen associated with acute hemorrhagic fever in Africa. Bas-Congo virus was added to the panel of viruses that samples were screened for in hemorrhagic fever cases.



Emerging Pandemic Threats PREVENT team interviewing villagers who survived an Ebola outbreak in the village of Mayibout II in the northeastern forest of Gabon.

In 2012, samples collected from several Bonobos in DRC, which had died from cardiopulmonary disease and hemorrhage, were sent to CIRMF for analysis. These specimens were screened for a broad range of pathogens and were found to be negative, except for one sample, which tested positive for adenovirus. The bacteriological analyses revealed that the Bonobos' deaths were likely the result of bacterial sepsis.

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In Indonesia, globalization and resulting changes in human activities, including wildlife trade, are increasing movement across and beyond the country.

PHOTOS BY NATIONAL ZOOLOGICAL PARK (LEFT) AND WILLIAM KARESH (RIGHT)

INDONESIA

Indonesia, a country of over 18,000 islands in equatorial Southeast Asia, has a wide range of habitats with abundant biodiversity, including endemic species of non human primates and rodents. Indonesia is considered a ‘megadiverse’ country and is home to numerous species of mammals. Bats alone account for 32% of the known mammalian diversity in the country (IUCN Red List 2014). The country’s biodiversity is at risk due to one of the most rapid rates of tropical deforestation globally, driven by increasing demands for food, forest products, and energy. These rapid ecological changes (e.g. clearing of peat swamp and tropical rain forests for oil palm plantations) are bringing humans into close contact with wildlife species that were previously rarely seen.

Indonesia has established large national parks with wildlife habitat to preserve its species diversity, and the country has an important ecotourism industry. In some parts of Indonesia, wildlife is an important source of protein, and people hunt and sell wild animals in markets and restaurants and also keep wild animals as pets. The wildlife trade, including human consumption of bats and rodents, is particularly widespread in areas of Northern Sulawesi. These human-wildlife interactions, many of which are growing through globalization, present opportunities for pathogen spillover from animals into people.

As an archipelago, Indonesia’s seas and oceans, in theory, may have historically limited spread of species of wild animals and the pathogens they may carry. However, globalization and resulting changes in human activities, including wildlife trade, are increasing movement across and beyond the country and therefore heightening the risk of cross-species transmission and the spread of pathogens. In addition, the potential for diseases to “spill-back” into animals from humans also enables greater potential pathogen spread and poses concerns for biodiversity conservation.

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Given the changing anthropogenic activities and the resulting increased contact between humans and wildlife in Indonesia, development of early warning systems, including expanded systematic surveillance and diagnostic capacity for potential zoonotic disease threats to people, was urgently needed. PREDICT-Indonesia was launched in the third year of the project with the goal of building an early warning platform to reduce the threat of zoonotic disease. Through a network of partnerships with scientific and government institutions, PREDICT worked to strengthen Indonesia's capacity to conduct disease surveillance, characterize zoonotic disease risks, and respond to zoonotic disease outbreaks.

PARTNERS

PREDICT-Indonesia managing institutions EcoHealth Alliance, Metabiota, and the Smithsonian Institution provided advisory support and regional coordination of activities in-country. Core PREDICT activities (program coordination, surveillance, animal testing, liaising with partners, and sharing of PREDICT protocols) were implemented by the Primate Research Center at Bogor Agricultural University. A close collaboration with the Eijkman Institute for Molecular Biology with support from the US Centers for Disease Control and Prevention, allowed for testing of archived human samples to complement animal testing.

A wide range of local partners supported activities and helped enable PREDICT and One Health achievements in the country. They were instrumental in advancing training opportunities, providing knowledge and personnel for surveillance efforts and strengthening national efforts to coordinate zoonotic disease detection and response.

Important partners included:

- USAID
- Ministry of Health
- Ministry of Agriculture
- Ministry of Forestry
- Coordinating Ministry of People's Welfare
- Indonesian Institute of Science (LIPI)
- KomNas Zoonosis Control
- Primate Research Center at Bogor Agricultural University
- Eijkman Institute for Molecular Biology
- Universitas Sam Ratulangi, Manado Sulawesi Utara
- Padjadjaran University, Bandung, Java

The PREDICT Indonesia team would like to acknowledge and express sincere appreciation for the support of Taman Safari/Bogor Botanical Gardens in the preparation and hosting of the PREDICT-Indonesia launch and training in 2012.

Endemic Indonesian primate species, *Macaca nigra*, at PREDICT field site in Sulawesi.



PHOTO BY WILLIAM KARESH



Colleagues from Institut Pertanian Bogor, EcoHealth Alliance, and University of California, Davis celebrate the launch of PREDICT Indonesia in January 2012. The training workshop and initial project launch were attended by over 20 participants from eight universities, government organizations, and research institutions which cover five regions of Indonesia: Aceh (northern part of Sumatra), East Java, Central Java and Yogyakarta, West Java, South Kalimantan, and North Sulawesi.

MAJOR ACHIEVEMENTS

- Assisted with development of the Emerging Virology Laboratory at Eijkman Institute (EVRUE) through improvements in infrastructure and personnel training (see Success Story for more information).
- Endorsed and contributed to an animal pathogen surveillance system for reporting to the national authority responsible for animal disease management. This system provides a coordinated mechanism for reporting animal diseases and improved flow of surveillance information within the country and the capacity to meet international reporting obligations to organizations such as the OIE and WHO.
- Assisted in the establishment of a National Committee for Wildlife Health, led by the Ministry of Forestry and Ministry of Agriculture. The Director General of Forest Protection and Nature Conservation decreed representation by PREDICT Indonesia on the committee.



PHOTO BY WILLIAM KARESH

Bats being prepared for sale in a market in Sulawesi.

- Collected samples from 421 animals among the three priority taxa (i.e. bats, rodents, and nonhuman primates) and obtained a suite of archived human and wildlife samples for diagnostic testing.
- Optimized 21 viral family testing protocols at the Primate Research Center, Bogor Agricultural University and Eijkman Institute laboratories for screening clinical specimens from wildlife and humans.
- In collaboration with FAO, PREDICT led a week-long laboratory training session for staff of Ministry of Agriculture at the Primate Research Center at Bogor Agricultural University. Trainees received hands-on training on using PREDICT diagnostic laboratory protocols to analyze poultry and other livestock samples for influenza viruses, paramyxoviruses, coronaviruses, and herpesviruses.
- Participated in development of a reporting system for human pathogen surveillance. Reports of activities and results are sent to the Director of the National Institute of Health Research and Development (NIHRD), the Center for Biomedical and Health Technology (at the NIHRD), the Eijkman Institute, and the National Commission of Zoonoses Control.
- In collaboration with Universitas Sam Ratulangi at Manado Sulawesi Utara, PREDICT conducted pathogen surveillance along the supply chain for the bat trade in Sulawesi and started to elucidate the bat bushmeat market chain through interactions with bat hunters and sellers. Together with the Emerging Pandemic Threats (EPT) PREVENT project, PREDICT also explored market dynamics in the country to better understand the zoonotic disease risks associated with market activities.

SUCCESS STORY

PREDICT assisted with development of the Emerging Virology Laboratory at Eijkman Institute (EVRUE) through improvements in infrastructure and personnel training. The laboratory was equipped with capabilities to test human samples for a broad range of viral families of emerging pandemic concern. The EVRUE team was increased to seven staff, all trained in advanced diagnostic methods relating to detection and characterization for emerging viruses.

This training provided EVRUE with the capacity to disseminate knowledge and techniques for enhanced emerging virus surveillance and diagnostics throughout Indonesia. PREDICT also contributed to upgrading capabilities at the Biotechnology Laboratory in the Bogor Agricultural Institute's Primate Research Center. Together with the center's Microbiology Laboratory, the Biotechnology Laboratory provides the testing for detection and characterization of a wide range of non human primate viral pathogens. With support from PREDICT-Indonesia, the laboratory also increased its capacity to detect novel pathogens from wildlife.

CAPACITY BUILDING

Developing Infrastructure

To improve laboratory capacity in Indonesia, PREDICT provided the EVRUE and IPB laboratories with equipment and supplies for detection, discovery, and characterization of pathogens with zoonotic potential in humans and wildlife.

PREDICT-Sponsored Training

PREDICT provided training to over fifty individuals representing government ministries, universities, and other scientific research institutions. The training included the following: analytical approaches that would be utilized in the PREDICT project, zoonotic pathogens of bats and rodents, human and animal safety during capture, bat and rodent sampling, sample collection and cold-chain, and laboratory safety and PPE.

The training was conducted through a number of different sessions, including a workshop where 20 attendees received training on One Health approaches, sampling protocols, and hands-on training in biosecurity and animal capture and sampling. The Emerging Virus Research Unit at Eijkman also actively held trainings for student, researcher, and laboratory staff from other laboratories in Indonesia. In collaboration with FAO, PREDICT led a week-long laboratory training for staff of Ministry of Agriculture at the Primate Research Center at Bogor Agricultural University. As part of the hands on training, staff analyzed poultry and other livestock samples for influenza viruses, paramyxovirus, coronavirus, and herpesvirus using PREDICT diagnostic laboratory protocols.

Emerging Virology Laboratory at Eijkman institute.



PHOTO BY ECOHEALTH ALLIANCE

PHOTO BY ADITYA PERKASA AND FRILASHITA AISYAH YUDAPUTRI



Participants at PREDICT training at LIPI Museum in 2012.



Panel discussion at the PREDICT Indonesia launch in 2012. PREDICT Co-Director Dr. Jonna Mazet joins colleagues from Government Ministries.

Partner-Organized Trainings

PREDICT staff also benefitted from trainings hosted by local and international partners. For example, EVRUE staff attended training sessions on laboratory maintenance and virology methodology provided by the WHO Reference Laboratory, manuscript writing provided by EPT RESPOND project, and arbovirus surveillance and diagnostics at the CDC.

Sharing Technical Expertise

PREDICT-Indonesia delivered presentations on surveillance, pathogen discovery, and biosecurity methods and best practices at a wide range of government meetings and scientific conferences.

SURVEILLANCE

PREDICT conducted surveillance at a range of sites across the country (Figure 1). These sites were characterized by diverse human-animal interfaces and species, including bats, rodents, and nonhuman primates. PREDICT also implemented viral pathogen screening of human samples from persons who had close contact with wildlife in several regions in the country.

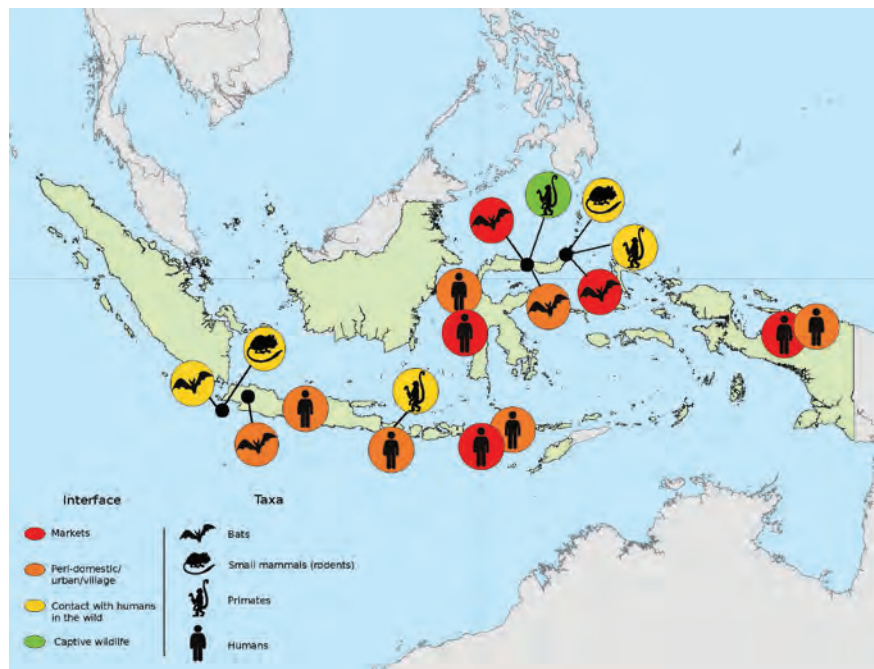


Figure 1. Sites where PREDICT conducted virus surveillance at high-risk disease transmission interfaces between wildlife and humans.

Sampling

During the three years that the PREDICT project was active in Indonesia, specimens were collected from 250 bats, 27 rodents, and 144 nonhuman primates (Figure 2). PREDICT conducted sampling at wildlife markets and at sites in diverse habitats throughout Indonesia, including urban areas and parks, lowland forests, montane rainforests, and coastal mangrove forests. In addition, bats and rodents from Tinjil Island, a semi-human modified site with a large introduced colony of long-tailed macaques (*Macaca fascicularis*), were also sampled in order to better understand cross-species transmission among the three target taxonomic groups.

Sampling was conducted at important high-risk disease transmission interfaces between animals and people, including large wildlife markets (i.e. > 20 vendors) with live and dead animals; ecotourism settings where there was contact between tourists and wildlife (mostly nonhuman primates); peri-domestic settings (i.e. in and around human dwellings/urban settings or villages); free-ranging wildlife in contact with researchers; and wildlife sanctuaries (Table 1). Wildlife wet markets and ecotourism sites were the focus of surveillance activities because of the high likelihood of close human-wildlife contact and potential for disease spillover at these interfaces.



PREDICT Indonesia field team member during a sampling expedition in Sulawesi.

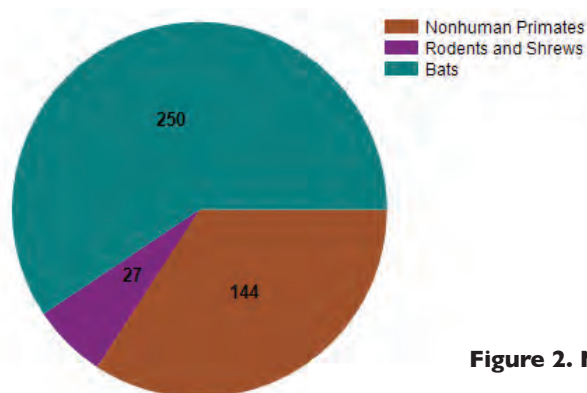


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Ecotourism and recreational activities	140	16	35	0
In or near human dwellings	0	0	37	0
Markets	0	0	158	0
Wildlife being studied	0	11	20	0
Zoos and sanctuaries	4	0	0	0
Total:	144	27	250	0



Human testing was performed at the EVRUE laboratory.

PREDICT-Indonesia's surveillance strategy included sampling designed to assess pathogen diversity at different stages of the wildlife trade in order to better understand where and when animals in the market chain are infected with or begin shedding viral pathogens. For example, fruit bats sold at a market in Gorontalo, Northern Sulawesi were traced back to a population of wild fruit bats at sites in a mangrove forest in Boalemo district. Sampling was conducted at the wet markets and at the remote locations where the source population was identified. Through these efforts, a novel paramyxovirus was found in bats that had been held in wet market conditions; however, the virus was not detected in the free-ranging source population.

In addition to wildlife pathogen surveillance, PREDICT-Indonesia conducted human disease surveillance through a risk-based approach. Priority samples and sample sets were identified through review of archival databases. EVRUE identified archived sample sets from cases of fever of unknown origin that tested negative for normative diagnostics (e.g. dengue fever and malaria) and individuals who were at a high risk for zoonotic pathogen exposure. Also, PREDICT obtained approximately 400 archived samples collected from populations in Bandung, Papua, Sulawesi, Java, Borneo, Bali, and Sumba that were found to have a higher risk of exposure to zoonotic pathogens. A number of these samples originated from febrile patients that had direct contact with wildlife through hunting and consumption and indirect contact with wild animals in and around their home.

Testing

Wildlife samples collected from PREDICT priority taxa were tested for arenaviruses, coronaviruses, filoviruses, flaviviruses, hantaviruses, henipaviruses, influenza, paramyxoviruses, poxviruses, retroviruses, rhabdoviruses, and seadornaviruses. Human samples were tested for seadornaviruses, paramyxoviruses, arenaviruses, hantaviruses, coronaviruses, henipaviruses, phleboviruses, filoviruses, rhabdoviruses, herpesviruses, orbiviruses, and enteroviruses.

DISCOVERY OF NOVEL VIRUSES INCLUDING A NEW PARAMYXOVIRUS IN FRUIT BATS

PREDICT-Indonesia detected a number of viruses in wildlife specimens, many of which were new. One notable finding was a new paramyxovirus found in fruit bats being sold in a wet market for food in Northern Sulawesi. Phylogenetic analysis places this new virus between the known viruses in the genera Rubulavirus and Respirovirus groups. There is currently no evidence this virus is zoonotic. Further characterization of the virus' genome is needed to determine if this virus may be transmissible to people or pose a risk to human health, as is more extensive testing of fruit bat specimens collected from this region to better understand the prevalence of the previously undescribed virus in wild free-ranging and market populations of fruit bats.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT-Indonesia assisted local and international partners in outbreak response and preparedness. Examples of outbreak efforts included:

- In response to H7N9 outbreaks in China, PREDICT-Indonesia attended government meetings to provide technical insight towards influenza virus preparedness.
- The PREDICT Indonesia Country Coordinator was invited by the Director of Animal Health Services to serve as an expert in a discussion regarding health requirements for nonhuman primate importation to Indonesia. This review led to a more standardized operating procedure for importation.
- PREDICT-Indonesia presented on project activities at a cross-sectoral meeting on laboratory diagnostics for zoonoses control, which was organized by the Ministry of Health, the Center of Research & Development for Disease Vector and Reservoir in Salatiga, supported by WHO, FAO, and USAID. Participants included government/national diagnostic labs for animal and public health, as well as universities and research institutes. PREDICT provided a source of technical support and expertise for the country's National Committee on Zoonoses and Emerging Infectious Diseases (KomNas Zoonosis Control).
- The Country Coordinator was appointed as a Member of the Expert and Academia Panel in the committee for the National Commission of Zoonosis Control to ensure representation from PREDICT.
- PREDICT-Indonesia also assisted the government with follow-up investigation on research conducted by an Indonesian scientist that suggested Ebola virus exposure in orangutans. The PREDICT Indonesia laboratories (IPB and Eijkman) confirmed through additional analysis on specimens remaining from the study that samples did not originate from orangutans as suggested in the published manuscript. The Ministry of Forestry's Directorate of Biodiversity Conservation and PREDICT wrote a letter to the journal editor to notify them of these findings.

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PHOTOS BY WILDLIFE CONSERVATION SOCIETY LAO PDR

In Lao PDR, wild animals are an important household food source in remote areas; however, wildlife populations are increasingly threatened by hunting for trade and loss of habitat as a result of logging and cultivation.

LAO PDR

Approximately 75% of emerging zoonotic diseases originate in wildlife (Jones et al. 2008). Activities that bring wildlife into close proximity with humans and livestock, such as wildlife trade, expanding human populations, and forest clearance, facilitate the opportunity for pathogen transmission. Wildlife trade has contributed to the emergence of a suite of diseases including SARS, monkeypox virus, and H5N1 influenza A (Guarner et al. 2004; Van Borm et al. 2005). Markets selling wildlife have acted as a source of previous disease outbreaks. In 2003, the SARS outbreak originated in a wildlife market in China and spread to 29 countries on five continents, leading to 8,098 human cases and 774 deaths worldwide and an estimated cost to China's economy of \$16.8 billion in lost tourism (Hai et al. 2004; Lam et al. 2003). Thus, wildlife trade and zoonotic diseases pose significant threats to human health, economies, and biodiversity in Southeast Asia.

Lao People's Democratic Republic (PDR) is a landlocked country in Southeast Asia with a population of 6,288,000 and a relatively low population density (27 people/km²) for this region (UN 2014). In 2002, the forest cover in Lao PDR was estimated at 41% (Bourgeois Luthi 2012), and there are currently 23 National Biodiversity Conservation Areas (NBCAs) and two corridors in Lao PDR, covering 3.5 million ha, which is equivalent to almost 15% of the country's land area (4th NRCBD report). Due to the country's low population and extensive forest cover, it is home to a rich diversity of wildlife, including mammal, reptile, bird, and amphibian species of national or global importance (Duckworth 1999).

Lao PDR is one of the poorest countries in which chronic malnutrition is high (Phengxay et al. 2007). Among non-Lao Tai ethnic groups, about 60% of children under five years of age are

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stunted, while 41% of children younger than five and 64% of children younger than two years of age suffer from anemia. Many also suffer from sub-clinical Vitamin A deficiency (Johnson et al., 2010; DOS and UNICEF 2008). In ethnic groups living in remote areas, wildlife can play a crucial household food source, and food security can be threatened by wildlife declines (Johnson et al., 2010). Thus, sustainably managing wildlife is important to both protect Lao PDR biodiversity and to reduce poverty and malnutrition in remote areas.

However, nearly all wildlife species are declining, and many are already at alarmingly low levels (Duckworth 1999; Johnson et al. 2010). Populations are increasingly threatened by high levels of hunting for domestic and international wildlife trade, as well as habitat loss, driven by shifting cultivation, logging for timber, and conversion of natural forests to cash crops and plantations at an unprecedented rate (Johnson et al. 2010; Duckworth 1999). Domestic wildlife trade in wet markets started in the 1980s and gained momentum after 1986, with the economic opening of the country following the inception of the New Economic Mechanism (Bourgeois Luthi 2012). Despite Lao PDR law prohibiting wildlife trade (Wildlife and Aquatic Law 2007), trade is common with wildlife being primarily sold for food and to a lesser degree for traditional medicine, pets, and for ornaments. The market availability of wildlife is also increasing due to the growing accessibility of wildlife areas resulting from road and infrastructure development and the opening up of wildlife areas due to illegal logging and other new activities (TRAFFIC 2008). The impacts of trade have been widespread, with large-sized mammals decreasing from the early 1980s and small mammals declining since the 2000s (Bourgeois Luthi 2012).

PREDICT and its partners in Lao PDR (i.e. Department of Livestock and Fisheries, including the provincial and district offices of Agriculture and Forestry; National Animal Health Laboratory; and the National Emerging Infectious Disease Coordination Office (NEIDCO)) built on previous partnerships between the human and animal health sectors that were developed during avian influenza outbreaks to expand the wildlife surveillance program and enhance capacity for early detection and response to emerging zoonotic pathogens in Lao PDR.



Wildlife trade in a market where the public come to buy, butcher then picnic on wildlife. Vendors openly sell both live and dead wildlife including flying squirrels, civets, wild birds, hares, and wild pigs.



Butchering of rodents in a rural village.

PARTNERS

PREDICT partners in Lao PDR included the Wildlife Conservation Society (WCS), USAID, and other partners:

- Department of Livestock and Fisheries, including provincial and district offices of Agriculture and Forestry
- National Animal Health Laboratory (NAHL)
- National Emerging Infectious Disease Coordination Office (NEIDCO)

MAJOR ACHIEVEMENTS

- Built national capacity for wildlife disease surveillance and viral detection to enable prompt response in the event of a disease outbreak (see Success Stories for more information).
- Conducted over 376 visits to 96 markets or roadside stalls across 15 provinces of Lao PDR where wildlife volume and species surveys were performed to identify key interfaces where wildlife and humans come into contact.
- Hosted workshops for central and provincial-level government officials from the Ministry of Health and the Ministry of Agriculture and Forestry. The workshops raised awareness of the One Health approach to zoonoses and emerging infectious diseases (EIDs) from wildlife and were attended by 54 provincial government officials.
- NAHL successfully implemented PREDICT's diagnostic laboratory protocols to test wildlife samples using broadly reactive consensus PCR assays.
- Collected samples from 2,693 wild animals from high-risk human-wildlife disease transmission interfaces across Lao PDR.
- Increased awareness on EIDs of wildlife-origin for 110 government personnel and eight students from the National University of Laos.
- Held One Health meetings with representatives from the central government and 12 provinces of Lao PDR to share results and recommendations for the project. In total, 95 people attended the meetings, including representatives from public health, agriculture and forestry, natural resource and environment, and industry and commerce. In addition, managers from markets that were found to be important wildlife-human interfaces were invited to the meetings.

SUCCESS STORY

Lao PDR Develops the Laboratory Capacity to Respond to Deadly Disease Outbreaks

Through the PREDICT project, Lao PDR developed in-country capacity to help detect wildlife pathogens and respond to potentially deadly disease outbreaks in ways that were not possible before. PREDICT developed innovative techniques for rapid detection and diagnosis of high-risk viral families through the use of synthetic DNA plasmids and broadly reactive consensus polymerase chain reaction (PCR). These techniques are powerful tools in the event of a disease outbreak. They



Staff at the National Animal Health Laboratory receiving training from a UC Davis laboratory technician on use of synthetic DNA plasmids and broadly reactive consensus PCR assays for viral diagnostics.

allow for rapid, broad screening to help rule in or out the involvement of viral pathogens in an outbreak. To develop these capabilities, a gradual and sustained capacity building program was undertaken with staff from the government animal laboratory and NAHL.

In November 2011, three NAHL staff members attended a month-long training at the Pasteur Institute in Cambodia. At this training, NAHL staff received training on personal protective equipment (PPE) use, laboratory biosafety, and protocols to extract DNA and RNA from samples. This capacity development was followed by a second month of training at the Pasteur Institute in June 2012, after which NAHL staff were capable of performing nucleic acid extraction of PREDICT samples.

In October 2012, a UC Davis laboratory expert conducted a one-month training session at NAHL on family level viral PCR for coronaviruses and rhabdoviruses, quality control, and cDNA production to allow shipping of stable testing products. Following this training, NAHL staff performed these protocols with limited ongoing assistance through weekly calls with UC Davis laboratory technical staff.

In-country PREDICT staff provided ongoing training and assistance with database and sample management. The collaboration between NAHL and the Pasteur Institute in Cambodia has allowed continued quality assessment of the PREDICT laboratory work at NAHL, with positive PCR samples sent to the Pasteur Institute for confirmatory testing.

To date, NAHL has performed RNA extractions and produced cDNA on more than 2,500 wildlife samples and conducted PCR on more than 700 samples. The provision of recurrent high quality training and application of techniques with consistent feedback to laboratory personnel has led to sustainable capacity for viral screening at NAHL, which is now prepared to assist in the event of a disease outbreak.

CAPACITY BUILDING

Increasing Awareness of Emerging Infectious Diseases

In July 2011, PREDICT hosted a workshop for central and provincial level government officials from the Ministry of Health and the Ministry of Agriculture and Forestry to increase awareness of EIDs. Twenty-five participants attended, including representatives from Houaphanh, Xiengkhuang, Vientiane, Bolikhamxay, Khammoune, Champassak, and Attapeu Provinces. The workshop covered a range of topics, including EIDs in wildlife, high-risk interfaces for disease transmission, surveillance for wildlife zoonoses in Lao PDR, risk mapping of high-risk

Workshop on emerging infectious diseases for central and provincial level government officials from the Ministry of Health and the Ministry of Agriculture and Forestry.

species and disease transmission interfaces, sample and data collection, wildlife handling and management, and use of PPE. At the end of the workshop, the attendees shared useful comments and information on human-wildlife risk interfaces.

Additional workshops to train greater numbers of provincial employees were requested. In response, PREDICT held three workshops in Bolikhamxay, Xieng Khouang, and Champassak Provinces for 54 provincial governmental officials from the Livestock and Fisheries Division, Forest Conservation Division, and Forest Inspection Division. The workshops raised awareness

of the utility of a One Health approach to detection, prevention, and control of zoonoses and EIDs from wildlife. Workshop participants were also provided training on proper use of PPE.

Building on the collaborative work between PREDICT and the Emerging Pandemic Threats PREVENT project to identify high-risk market interfaces in Lao PDR, PREDICT staff were invited to present on wildlife-origin EIDs at market stakeholder meetings in Vientiane and Vang Vieng in order to increase awareness among market owners and provincial and district public health staff regarding the zoonotic disease risk associated with wildlife markets.

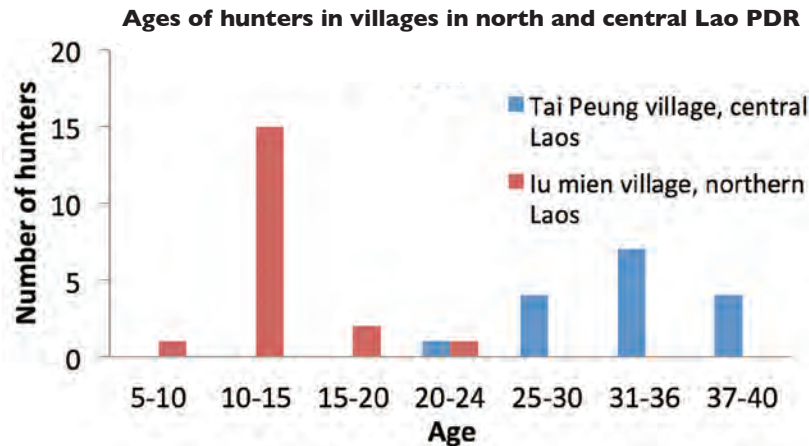


Developed National Capacity for Field Surveillance of Wildlife Zoonoses

Training of Trainers. During the course of the PREDICT project, national capacity was significantly increased for wildlife disease surveillance. Two team members received extensive training on wildlife disease surveillance techniques and database management in order to in-turn provide knowledge and tools for national government staff and university students. In addition to education on EIDs and training on field anesthesia and wildlife surveillance methods, staff members also received unique training on wildlife necropsy techniques at the Bronx

Zoo, sample bar-code systems, observational wildlife trade survey techniques, human-animal exposure survey techniques, and statistics. The PREDICT staff provided field-based training for NAHL staff on wildlife surveillance techniques and assisted NAHL with management of their sample database and specimen tracking. In addition, they supervised PREDICT student thesis projects on wildlife hunting practices, assisting them with project design and analysis. PREDICT Lao PDR staff also co-presented on EIDs at provincial government workshops, the 2013 Wildlife Health Forum in Bangkok, and the One Health symposium in Lao PDR in 2013.

Provincial workshops on emerging infectious diseases held in Bolikhamxay, Xieng Khouang, and Champassak provinces.



Data collected by students trained and funded by PREDICT who undertook their final year thesis projects in hunting villages in rural Lao PDR.

Training of Government Staff. Over eight months of collaborative field work was performed by staff from PREDICT and NAHL, the government body responsible for animal disease outbreak response. On each field trip, NAHL staff members accompanied the PREDICT team and received field based training on use of PPE, safe wildlife sampling, wildlife species identification, sample labeling, cold chain maintenance for viral samples, and sample transportation. Eight NAHL staff members were trained, and the agency is now capable of conducting wildlife zoonoses surveillance independent of the PREDICT team. PREDICT also supported the participation of NAHL staff in the first regional wildlife pathology workshop in Vietnam, as well as biological safety cabinet and laboratory fume hood training sessions.

Training of University Students. Over three years, eight students from the National University of Laos were trained by the PREDICT team on observational survey methods, PPE, wildlife sampling techniques, and sample storage. PREDICT then supported these students in their efforts to conduct research in wildlife hunting villages for their thesis projects. In addition to training the next generation of scientists, valuable information was obtained on wildlife hunting and consumption practices, which can be used for zoonotic risk mitigation strategies.

Established Diagnostic Capacity at the National Animal Health Laboratory for Viral Family Testing on Wildlife Samples

Capacity was developed at NAHL for testing viral families using synthetic DNA plasmids and broadly reactive consensus PCR assays. Multiple training sessions at Pasteur Institute of Cambodia and an in-house session provided by UC Davis laboratory personnel has allowed NAHL personnel to independently conduct RNA extraction, cDNA production, viral family level PCR for coronaviruses and rhabdoviruses, and quality control assessments.

SURVEILLANCE

Samples have been collected from priority taxonomic wildlife groups (i.e. bats, nonhuman primates, and rodents) at high-risk human-wildlife disease transmission interfaces across 14 provinces of Lao PDR, focusing on the wildlife trade (i.e. markets and roadside stalls selling wildlife), subsistence hunting in indigenous villages, captive wildlife collections in zoos or held

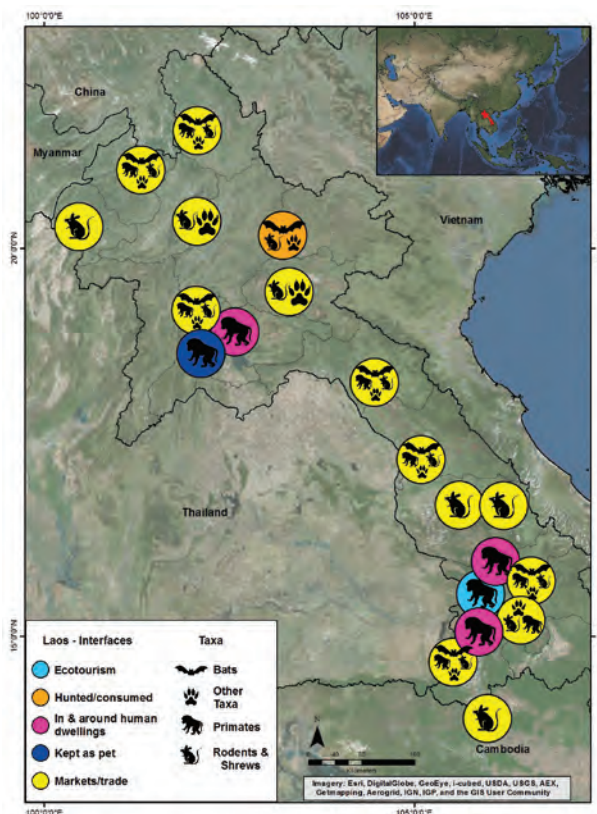


PHOTO BY WCS LAO PDR

The Lao PDR PREDICT team conducted sampling of bats, rodents, and primates across the country. This photo shows the team sampling a macaque.

as private pets, wildlife in and around human dwellings, and ecotourism sites (Figure 1). A total of 2,693 animals (1,530 rodents, 956 bats, 45 primates, and 162 from other species, including civets) were sampled from these high-risk interfaces (Figure 2 and Table 1). Samples were screened using consensus PCR for alphaviruses, arenaviruses, astroviruses, coronaviruses, filoviruses, flaviviruses, hantaviruses, henipaviruses, herpesviruses, influenza viruses, rhabdoviruses, lyssaviruses, seadornaviruses, paramyxoviruses, poxviruses, bunyaviruses, and retroviruses.

Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.



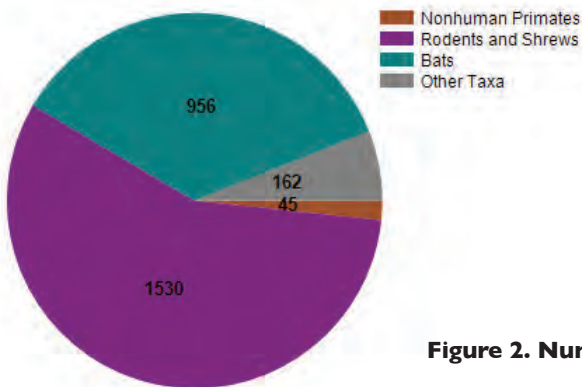


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Ecotourism and recreational activities	1	0	0	0
In or near human dwellings	6	0	0	0
Hunted Wildlife	1	408	125	22
Markets	20	1118	831	138
Private sale	0	4	0	2
Zoo and sanctuaries	17	0	0	0
Total:	45	1530	956	162

Market Surveys

Over a 3.5 year period, 376 market surveys were conducted, each survey consisting of one visit to a market or roadside stall. Two field staff were trained in observational market survey techniques. At each market or roadside stall, surveyors recorded the GPS position, the species of animals for sale, the body part type being sold, the number of carcasses or estimated weight of body parts being sold, the condition of the item (live, dried, fermented, fresh, frozen, pickled, or smoked), and sale price.

Detailed biosecurity and shopper origin surveys were conducted in collaboration with PREVENT over a three-month period. These more detailed surveys were conducted once per market, and general characteristics of the market were recorded, such as license plates observed on vehicles near the market (to assess shopper origin), observation of foreigners shopping in the market, volume of domestic animals, and general hygiene throughout the market. Observations on wildlife vendor and butcher hygiene was limited to observation of one wildlife vendor and one wildlife butcher (if present) per day for 30 minutes.



Wildlife trade in markets: a vendor selling bats.



Wildlife trade in markets in Lao PDR: squirrels, flying squirrels, mouse deer, and civets being sold.

Characterization of the Wildlife Trade

Through work undertaken by PREDICT, we now have a better understanding of the scale of the wildlife-human contact interface in markets. Over 376 visits to 93 markets or roadside stalls during the PREDICT project, 17,756 wild birds, 11,554 mammals, and 448 reptiles were observed. Mammal observations included 6,862 rodents, 3,638 bats, 58 primates, and 2,151 kg of ungulates. These results represent a fraction of wildlife trade in Lao PDR and demonstrated the high level of human exposure to wildlife that is occurring. Overall, trends showed that freshly killed wildlife was more commonly sold compared to live wildlife (30% of rodents, 10% of wild birds, 4% of bats, and 53% of primates observed at sale were alive). However at certain markets, the number of live animals at the time of sale was relatively high. For example, of the 4,526 wild animals observed during sale over five visits to a market in southern Lao PDR, 85% of the individuals were alive (mostly rodents and lizards). In addition, at a market on the main northern transport route, of the 677 wild animals observed, 82% were sold alive (mostly wild birds).

Biosecurity surveys in markets undertaken by the PREDICT team in collaboration with PREVENT, highlighted the occurrence of high-risk behaviors for pathogen contamination, such as vendor butchering of wildlife combined with absence of basic sanitary measures (i.e. hand washing and table cleaning). Buyers at markets were not only locals, but also persons from other provinces and other countries. For example, customers at the market in southern Lao PDR often came from Thailand to purchase, butcher, and consume wildlife. In addition, the demand for wildlife in Lao PDR by urban populations as a luxury food item is widespread (Singh 2008; pers comm. Lao PDR government officials). Therefore, as wildlife is increasingly purchased and transported to densely populated urban locations to meet this demand, the risk of pathogen spread to wider populations increases.

During sampling trips by the PREDICT team in rural Lao PDR hunting communities, information was gathered on high-risk behaviors, including wildlife butchering with poor biosecurity practices, consumption of large numbers of rodents and bats in areas where larger wildlife has been overharvested, and consumption of raw or partially cooked wildlife, including nonhuman primates. Furthermore, hunting villages also kept primates alive at the village prior to sale or consumption.

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PHOTOS BY TOM HUGHES (LEFT) AND JIMMY LEE (RIGHT)

In Malaysia, anthropogenic land-use change, including the expansion and intensification of livestock production, has brought human settlements and commercial agriculture into proximity with wildlife.

MALAYSIA

Countries in Southeast Asia rank among the highest in the world for biodiversity yet have experienced some of the most rapid deforestation. Over the past few decades, oil palm has been one of the most rapidly expanding crops in the region (Koh and Wilcove 2008). Malaysia is the second largest oil palm producer in the world, and much of the deforestation in the country has occurred as a result of land conversion for palm oil plantations. Anthropogenic land-use change, including the expansion and intensification of livestock production, has brought human settlements and commercial agriculture into proximity with wildlife. In Malaysia's villages, there are few or no barriers between people, their domestic animals, and wildlife.

Malaysia has been identified as a hotspot for zoonotic disease emergence and is where Nipah virus, a deadly paramyxovirus carried by pteropid fruit bats, first emerged in 1998 (Chua 2003; Halpin et al. 2011). The first emergence of Nipah virus occurred on a large-scale pig farm on the outskirts of Malaysia's fifth largest city Ipoh in the state of Perak approximately 200 km north of the capital Kuala Lumpur. The intensification of swine production, coupled with the presence of cultivated fruit orchards on the farm, allowed for the spillover of Nipah virus from bats into pigs and humans. Its subsequent circulation ignited the epidemic that ultimately killed nearly half of the 200 infected farm workers and had a devastating impact on the swine production industry in Malaysia (Pulliam et al. 2012). The high degree of contact among people, livestock, and wildlife in Malaysia contributes to its shared status among countries highly vulnerable to zoonotic disease emergence, and therefore Malaysia is a country prioritized for development of a coordinated national wildlife disease surveillance system (Jones et al. 2008).

Because of the cross-sectoral impact of zoonotic diseases involving wildlife, livestock, and people (e.g. Nipah virus, HPAI H5N1, and Middle East Respiratory Syndrome (MERS) Coronavirus),

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prevention and control of zoonotic pathogens can be substantially enhanced by the coordination of efforts from ministries of health, agriculture, and environment (the wildlife authority). The utility of a One Health approach for infectious disease research, prevention, and control is increasingly recognized among scientists and some government agencies globally, but divisions among ministries persist and make coordinated surveillance activities difficult to realize. Malaysia has had firsthand experience responding to a novel zoonotic disease outbreak. During the 1998 Nipah outbreak, the Malaysian Government established the Inter-Ministerial Committee for Zoonotic Disease Control. This inter-ministerial platform was an important factor in controlling the Nipah virus outbreak, which required close coordination among human and animal health agencies. However, in the years following the Nipah outbreak, the Inter-Ministerial Committee for Zoonotic Disease Control stopped meeting, and there was no regular communication among livestock, wildlife, and health departments outside of emergencies.

In 2009 and 2010, PREDICT began to discuss the need to re-establish this inter-ministerial platform to facilitate the integration of wildlife disease surveillance into the public health infrastructure. In part due to PREDICT's efforts, and in recognition of the growing importance of the One Health concept, the Inter-Ministerial Committee for Zoonotic Disease Control began to meet regularly again. In addition to government partners, local universities involved in zoonotic disease surveillance are invited to attend and share their research. PREDICT attended several of these meetings to inform members of the committee of our progress.

In 2012, PREDICT, the Ministry of Health, the Department of Veterinary Services, and the Department of Wildlife and National Parks also established the Zoonosis Technical Working Committee. This cross-sectoral network convenes to deal with human and animal health issues, including mitigation strategies for decreasing the risk of emerging infectious diseases, and reports to the Inter-Ministerial Committee for Zoonotic Disease Control. PREDICT also increased capacity within a laboratory network that exchanged sample testing techniques, technologies, and personnel to enhance disease detection and wildlife surveillance capacities in Malaysia. These platforms enhanced the Government of Malaysia's ability to detect and respond to zoonotic pathogens.

Partners

PREDICT partners in Malaysia included EcoHealth Alliance (EHA), the Center for Infection and Immunity (CII) at Columbia University, Global Viral, USAID, and the Smithsonian Institution.

Malaysian partners included:

- Department of Wildlife and National Parks (DWNP)
- Department of Veterinary Services (DVS)
- Veterinary Research Institute (VRI)
- Ministry of Health (MoH)

Bat bags at PREDICT's Deep Forest sampling sites in Gomatong Forest Reserve, Sabah.



PHOTO BY TOM HUGHES

- Department of State Health Sabah (DSHS)
- Sabah Wildlife Department (SWD)
- Danau Girang Field Centre (DGFC)

MAJOR ACHIEVEMENTS

- Expanded the One Health Workforce by forming the Zoonosis Technical Working Committee with the Department of Veterinary Services (DVS), Ministry of Health (MoH), and the Department of Wildlife and National Parks (DWNP; see Success Stories for more information).
- Trained 21 DWNP officers from states where PREDICT sampling was conducted. PREDICT and DWNP sampled 1,063 animals in Peninsular Malaysia.
- Helped DWNP establish a dedicated surveillance team to conduct routine surveillance and respond to outbreaks. The DWNP-dedicated surveillance team independently sampled an additional 423 animals for PREDICT and another 313 outside of the PREDICT project in Peninsular Malaysia following PREDICT protocols.
- Provided good laboratory practices and biosafety training to 45 staff from DWNP, VRI, and SWD. In total, PREDICT trained 130 individuals from government partners, local universities, and NGOs in surveillance and diagnostic techniques including sharing protocols.
- Surveyed priority wildlife taxa within pristine, semi-disturbed (agricultural), and disturbed landscapes along the lower Kinabatangan River during the wet and dry seasons for the Deep Forest study.
- Coordinated with the Department of State Health Sabah and Sabah Wildlife Department (SWD) to conduct behavioral surveys to measure and characterize human-wildlife contact at the Deep Forest sites in Sabah. PREDICT surveyed 406 villagers, oil palm workers, and tourists.
- Created the Wildlife Health Unit, a dedicated surveillance team, with Sabah Wildlife Department. The Wildlife Health Unit sampled 1,179 animals in Sabah from 2012 to 2014.

Traveling along the Kinabatangan River evaluating potential Deep Forest sites.



PHOTO BY TOM HUGHES

- Helped create BSL-2 molecular diagnostic laboratories at DWNP, VRI, and the Wildlife Health Unit in Sabah for livestock and wildlife pathogen testing.
- PREDICT and the MoH screened 136 archived samples from Orang Asli community (indigenous population) patients with acute febrile illness at the National Public Health Laboratory (NPHL) using PREDICT PCR universal controls and viral family protocols.
- Tested 8,324 samples from 2,665 animals from Peninsular Malaysia and Sabah at DWNP, VRI, WHGFL, and CII using PREDICT PCR and other protocols.

SUCCESS STORY

Expanding the One Health Workforce

Although Malaysia is considered a hotspot for zoonotic disease emergence, infrastructure for detecting novel and potentially zoonotic wildlife pathogens was largely lacking in-country prior to implementation of the PREDICT project. In addition, since the 1998 Nipah virus outbreak there had been little collaboration in Malaysia across sectors with expertise in human, domestic animal, and wildlife health; ecology; and laboratory sciences. While the utility of a One Health approach for infectious disease research, prevention, and control is increasingly recognized among scientists and some government agencies globally, divisions among ministries persist, making coordinated surveillance activities challenging.

One of PREDICT-Malaysia's first tasks was to help the Malaysian Government recognize the importance of the One Health concept and the need for regular communication among the human, wildlife, and livestock sectors. Using Malaysia's experiences with Nipah virus as an example, PREDICT-Malaysia was able to highlight the need for regular cross-sector communication, which resulted in the re-establishment of the Inter-Ministerial Committee for Zoonotic Disease Control. PREDICT Malaysia then established the Zoonosis Technical Working Committee with the Department of Veterinary Services, Ministry of Health, and Department of Wildlife and National Parks (DWNP) to strengthen a national network for wildlife health and diagnostics. Traditionally, DWNP had been excluded from many discussions related to disease and wildlife surveillance. Involvement with PREDICT has helped to strengthen their capacity and role with regard to disease surveillance. For the first time since the Nipah outbreak, samples collected by DWNP have been screened at Veterinary Research Institute (VRI), and communication and collaboration between these departments has significantly improved.

In addition, PREDICT engaged DWNP and Sabah Wildlife Department in the development of a cadre of wildlife officers who are skilled in the safe capture, handling, and sampling of wildlife. Laboratory capacity was built within these departments to screen wildlife samples using broad viral family-level PCR assays. The laboratories are linked to the VRI, under the Department of Veterinary Services (DVS), which strengthened the connection between wildlife and livestock departments. Results from laboratory testing at DWNP and VRI were regularly shared with the Zoonoses Technical Working Committee, which included representatives from DWNP, DVS, and the Ministry of Health, ensuring that all three sectors were informed about novel viruses discovered at high-risk disease transmission interfaces. Training on surveillance and laboratory diagnostic techniques was provided to 130 individuals from government partners, local universities, and NGOs. This training allowed for integration of wildlife surveillance into standard operations at DWNP and SWD and contributed to the development of a One Health workforce.

In Sabah, PREDICT worked with the Sabah Wildlife Department and Danau Girang Field Centre (DGFC) to establish and develop the

The Wildlife Health Unit included wildlife officers from SWD Wildlife Rescue Unit and DGFC who were trained by PREDICT to be able to safely and effectively conduct wildlife disease surveillance.



PHOTO BY TOM HUGHES

Wildlife Health Unit (WHU), a new division within the Sabah Wildlife Department's Wildlife Rescue Unit that is specifically dedicated to disease surveillance activities and managed by SWD and PREDICT-Malaysia's Country Coordinator. The unit expands the technical expertise of wildlife rangers to include disease surveillance and has provided significant opportunities for professional development and training on topics, such as biosafety and safe wildlife handling and sampling for zoonotic agents, optimal sample handling and analysis, and molecular data analysis.

PREDICT also conducted 32 training sessions for participants from DWNP, NPHL, VRI, SWD, University Putra Malaysia, WWF Malaysia, DGFC, Sepilok Orangutan Rehabilitation Center, Borneo Sun Bear Conservation Centre, and the Institute for Tropical Biodiversity and Conservation, University Malaysia Sabah in order to build working relationships and strengthen communication among government departments, NGOs, and universities working with wildlife and/or zoonotic diseases on Peninsular Malaysia and in Sabah. Improvements in wildlife disease surveillance capacity within the DWNP resulted in a dedicated and self-sufficient team, which received funding from the Ministry of Natural Resources and Environment to support ongoing wildlife surveillance activities. This development was an important step towards connecting wildlife health experts to livestock and human health experts, thereby expanding and improving the national One Health workforce.

CAPACITY BUILDING

Surveillance Improvements

PREDICT helped to establish a consistent cold chain during surveillance activities, using portable liquid nitrogen vapor containers to maintain samples at ultra-cold temperatures in the field and during transport to the three partner laboratories (DWNP Headquarters, Wildlife Health, Genetics and Forensics Laboratory, Sabah, and VRI). Laboratory freezers were augmented with backup systems and mobile phone alarm systems in case of power or freezer failure.

Staff from Sabah Wildlife Department and Danau Girang Field Centre sampling a Proboscis monkey.

Laboratory Capacity

PREDICT enhanced laboratory capacity in Sabah through establishment of the PREDICT/SWD/DGFC Wildlife Health, Genetic and Forensic Laboratory (WHGFL) in Sabah, a BSL-2 laboratory accredited and certified in accordance with CDC and NIH laboratory standards.

The lab was used to conduct health checks on rescued and relocated wildlife before being released into new areas or sanctuaries, to screen samples for PREDICT and Deep Forest, and for genetic research and forensic investigations. This was SWD's first laboratory and the first certified BSL-2 laboratory dedicated to wildlife surveillance in Malaysia.

In addition, PREDICT helped create an animal processing and sample containment room at DWNP and refurbished a dedicated BSL-2 molecular diagnostic laboratory for viral pathogen testing next to the existing wildlife forensics and genetics lab. This



PHOTO BY TOM HUGHES

laboratory was the first-ever dedicated disease diagnostic laboratory at the Wildlife Department's headquarters. The lab and animal processing room at DWNP served as a training center for wildlife officers and laboratory personnel.

Department of Wildlife and National Parks used findings from the PREDICT screening at this lab to strengthen their proposal for funding for a new laboratory complex and to highlight the importance of improving laboratory capacity for wildlife disease surveillance. In 2013, DWNP received substantial new funding from the Ministry of Natural Resources and Environment to create a free-standing disease diagnostics and forensics laboratory on site which will come online in 2015. This laboratory will create new jobs for molecular biologists in the wildlife conservation and health sector, which will ultimately help to expand the One Health workforce. This proposal had been rejected on five previous submissions, and its approval was in no small part due to PREDICT development of the laboratory and disease findings that allowed DWNP to justify the expense of laboratory development and show the Ministry that they had the human capacity to carry out surveillance activities.

Through a partnership with the VRI in Ipoh, PREDICT helped design and equip a BSL-2 molecular diagnostic lab within the new BSL-3 agriculture building. This lab is used to screen livestock samples as part of VRI's routine disease surveillance but was also used for testing wildlife samples for PREDICT. VRI was a vital partner for PREDICT, providing important laboratory infrastructure and, with the Department of Wildlife and National Parks, helped PREDICT achieve the aims of the EPT program.

Each partner laboratory in Malaysia was provided reagents and primers and a universal positive control that can be used to screen for 17 viral families. PREDICT assisted with development of quality assurance and quality control standard operating procedures. PREDICT developed all standard operating procedures (SOPs) and protocols for WHGFL and helped to improve and standardize protocols and SOPs for the three laboratories it helped develop.

Mei-Ho Lee prepares wildlife samples for extraction at the BSL 2 molecular diagnostic lab PREDICT helped refurbish at the Wildlife Department's headquarters.



PHOTO BY JIMMY LEE

Training

PREDICT trained 130 staff from DWNP, NPHL, VRI, SWD, and various NGOs and universities in Malaysia. Training focused on field techniques for safe wildlife capture, handling, sampling, and sample transport from field to lab; laboratory techniques including molecular virology; and laboratory management and safety training. Further, PREDICT trained 21 DWNP officers from states on Peninsular Malaysia where PREDICT sampling was conducted. The DWNP established a dedicated surveillance team to conduct routine surveillance and respond to outbreaks.



FAO Regional BioSafety Coordinator, Dr. Stuart Blacksell, conducting spill clean up training at the Veterinary Research Institute.

PHOTO BY TOM HUGHES

PREDICT conducted four weeks of advanced laboratory training at Columbia University's Center for Infection and Immunity, providing hands on training in high throughput sequencing (application of rapid technology to screen large amounts of genetic material) and PREDICT PCR protocols. Two members of the PREDICT Malaysia lab team: a geneticist from DWNP and the PREDICT molecular biologist travelled to New York to process wildlife samples using these techniques. High throughput sequencing was not widely available in Malaysia, but a few institutions were beginning to use this technology, so the skills will be transferrable to in-country work in the future.

SURVEILLANCE

In East Malaysia, PREDICT partnered with Sabah Wildlife Department (SWD) and Danau Girang Field Center. Through this partnership, intensive surveillance was carried out for the Deep Forest study (see section on Deep Forest), a coordinated study in three of the most pristine forests in the world: the Amazon Rainforest in Brazil, the Bwindi Impenetrable Forest in Uganda, and the Lower Kinabatangan River Basin in Sabah.

The Deep Forest study in Sabah was designed to provide information to PREDICT and the government of Malaysia about the effects of land conversion on the risk of zoonotic disease emergence from wildlife. The aims of the surveillance effort were to compare the abundance of key wildlife hosts (bats, rodents, and nonhuman primates) along a land-use gradient shifting from pristine forest to agricultural land to heavily populated (urbanized) environments (Figure 1) and to assess the impacts of land-use change on viral diversity and wildlife host assemblages along a deforestation gradient. Sampling was conducted at all three sites in both the dry and wet seasons, and this survey was aligned with the two parallel efforts in Uganda and Brazil.

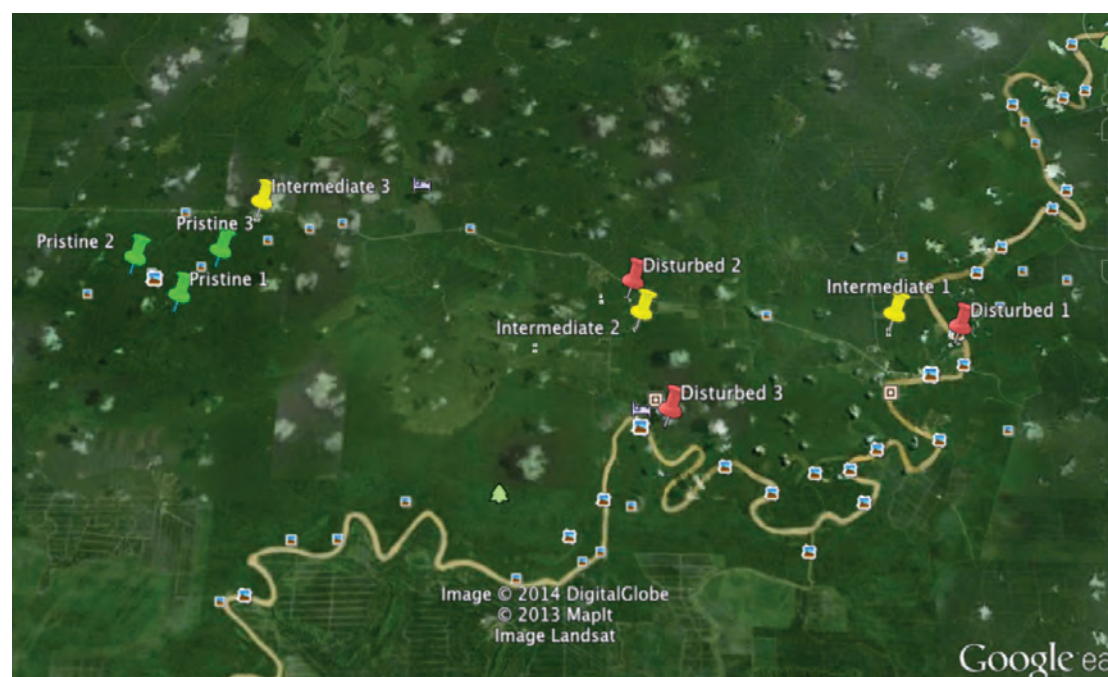


Figure 1. Map of the lower Kinabatangan River showing Deep Forest field sites across a land-use gradient. The Deep Forest surveillance design was standardized across the land-use gradient, and included three different survey sites in each environment. Bats, rodents, and nonhuman primates were sampled at each site and screened for novel viral agents.

Surveillance was also targeted at important high-risk disease transmission interfaces between wildlife and humans, including wildlife in contact with park personnel and workers harvesting crops, wildlife in and around human dwellings or agricultural fields, and wild animals in the wildlife trade and in sanctuaries (Tables 1 and 2).

- Across Malaysia, more than 2,300 animals from priority taxa were sampled (Figure 2). In total, samples from 2,665 animals (Figures 3- 4; comprised of 119 species have been screened to date for 17 viral families, including 14 viral families of public health importance (adenoviruses, astroviruses, bunyaviruses, coronaviruses, filoviruses, flaviviruses, henipaviruses, herpesviruses, influenza viruses, orthopoxviruses, paramyxoviruses, parapoxviruses, rhabdoviruses, and seadornaviruses). Samples were collected using the noninvasive, nonlethal PREDICT protocols and were tested for a panel of potential pathogens at VRI, WHGFL, DWNP, and CII. Screening at CII, DWNP, VRI, and WHGFL has identified a number of known and new viruses.

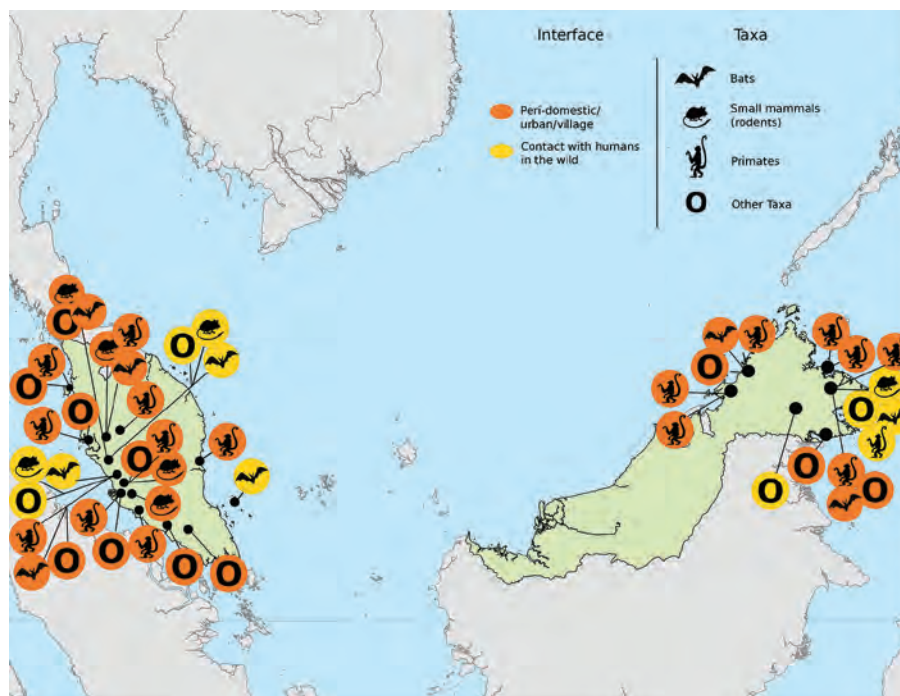


Figure 2. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

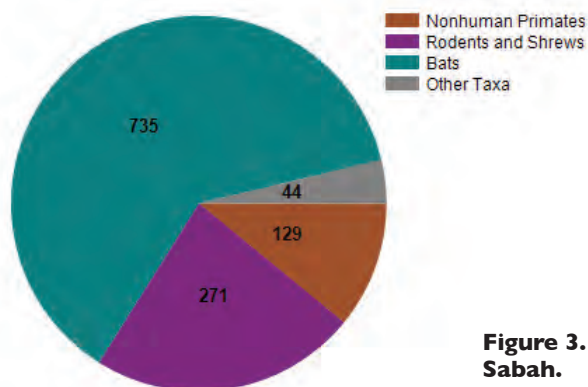


Figure 3. Number of animals sampled by taxa, Sabah.

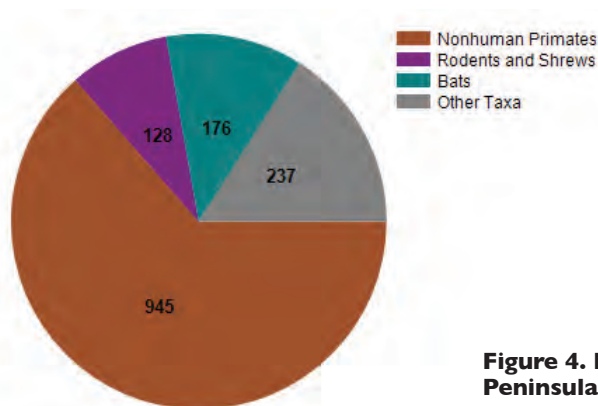


Figure 4. Number of animals sampled by taxa, Peninsular Malaysia.

Table 1. Number of animals sampled according to targeted transmission interfaces, Sabah.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	14	181	254	22
Ecotourism and recreational activities	19	0	0	0
In or near human dwellings	5	26	30	2
Wildlife being studied	7	0	0	0
Protected areas	71	64	450	15
Zoos and sanctuaries	13	0	1	5
Total:	129	271	735	44

Table 2. Number of animals sampled according to targeted transmission interfaces, Peninsular Malaysia

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	197	18	8	2
Ecotourism and recreational activities	4	16	44	15
In or near human dwellings	726	16	65	9
Wildlife trade	13	0	0	190
Protected areas	0	77	59	7
Zoos and sanctuaries	5	1	0	14
Total:	945	128	176	237

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT trained DWNP and SWD surveillance teams on outbreak response preparedness and provided PPE and protocols to assist with outbreak response preparation. At the request of the MoH and Department of State Health in Sabah, PREDICT provided advice and assistance during a number of disease outbreaks, including sarcocystosis and leptospirosis.

In addition, Sabah Wildlife Department requested assistance from PREDICT with a pygmy elephant die-off in Sabah. PREDICT PCR protocols were used to rule out infectious disease. With coordination through USAID RDMA, the PREDICT team in Malaysia arranged for samples to be screened at AFRIMS, the Ramathibodi Poison Center in Thailand, and Queensland Biosecurity Sciences Laboratory in Australia.

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Mexico's high biodiversity and natural systems have been altered dramatically by human activities in the past decades with potential effects on the likelihood of contact between wildlife, livestock, and people.

MEXICO

Mexico spans two biogeographic regions, the Nearctic and Neotropics, and ranks among the top ten countries for species richness (Martinez-Meyer et al. 2014). Its conical shape forces the convergence of major migratory corridors for many taxonomic groups, including mammals, birds, and reptiles. Due to its unique geological and climatic history, it represents less than 1% of the earth's surface, yet holds 5-10% of the world's biodiversity, including many endemic species (Mittermeier et al. 1997). Mexico's natural wealth is also reflected by the great diversity of ecological processes, which have been altered dramatically by human activities in the past decades. Natural vegetation in Mexico continues to diminish; by 2002 it had been reduced to only 38% of its original area in the country (Sarukhán et al. 2009).

Anthropogenic impacts, such as land-use change, lead to perturbations of natural systems with potential effects on the likelihood of contact among wildlife, livestock, and people. Mexico is considered a hotspot for emerging infectious diseases (EIDs) of wildlife origin because of its high biodiversity and dense human population (Jones et al. 2008; Morse et al. 2012). Its potential as an EID hotspot has been illustrated by recent outbreaks of disease in people and wildlife, including pandemic H1N1 influenza virus, West Nile virus and other flaviviruses, coronaviruses, alphaviruses, and hantaviruses (Zepeda-Lopez et al. 2010; Anthony et al. 2013; Mann et al. 2013; Sotomayor-Bonilla et al. In Press). These events and their potential connection to land-use change and biodiversity have revealed the need to understand the relationships between pathogens, reservoirs (including people), and vectors (mosquitoes and ticks) in Mexico. Mexico's unique biogeography and diversity make it an ideal location to investigate some of the most important questions regarding drivers of disease emergence.

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PARTNERS

PREDICT partners in Mexico included EcoHealth Alliance (EHA), Facultad de Medicina Veterinaria y Zootécnica (FMVZ) and Instituto de Ecología, Universidad Nacional Autónoma de México (UNAM), USAID, and the Center for Infection and Immunity (CII) at Columbia University.

Other local partners included:

- Kalaan-Kab AC
- BIOCONCIENCIA
- Laboratorio de Medicina para la Conservación, Instituto Politécnico Nacional/Conservation Medicine Lab (IPN)
- Instituto Mexicano del Seguro Social (IMSS)
- Consejo Técnico Consultivo Nacional de Sanidad Animal/Technical Council of Animal Health (CONASA)
- Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación/Mexican Department of Agriculture (SAGARPA)
- Comisión Nacional de Salud Animal/Wildlife Health Commission
- Comisión Nacional de Áreas Naturales Protegidas/Commission for Protected Natural Areas (CONANP)
- Secretaría de Medio Ambiente y Recursos Naturales/Secretary of the Environment and Natural Resources (SEMARNAT)

MAJOR ACHIEVEMENTS

- Integrated research on human and animal systems to develop a One Health framework for the PREDICT project in Mexico (see Success Stories for more information).
- Met the major country goals for capacity building set by the Emerging Pandemic Threats program for PREDICT in the two-and-half-years that the project was active in Mexico.
- Detected novel viruses in bats, including novel herpesviruses, astroviruses, and coronaviruses.
- Trained 28 people (54% women) in core safety; animal capture, handling, and sampling skills; lab safety; PPE use and biosafety; zoonotic disease risks; disease modeling; animal care and use protocols; and lab diagnostic techniques.
- Collected samples from 2,028 animals, 99% of which came from high-risk taxa groups (i.e. bats and rodents).
- Provided PREDICT protocols, positive controls, and primers to laboratories in Mexico City (IMSS, UNAM, and IPN). All laboratories are now equipped and have the technical expertise to conduct family level viral diagnostics on clinical samples.

SUCCESS STORY

Development of One Health Framework

The PREDICT Mexico team integrated research on human and animal systems to develop a One Health framework for the program in Mexico. The team's efforts resulted in two successful international conferences, several well-trained Master's and PhD students, and the establishment of a wildlife health position on Mexico's Technical Council of Animal Health.

Initiating Discussions. The International Conferences of Disease Ecology and Conservation Medicine were hosted by the PREDICT team along with Mexican universities and the non-profit Kalaan-Kab AC (Mexican Association of Conservation Medicine). The conferences, in 2011 and 2013, drew more than 150 attendees each from several organizations, including EcoHealth Alliance, UC Davis, Columbia University, the Smithsonian Institution, and the London Zoological Society. The conferences highlighted the importance of a One Health approach to zoonotic diseases and developing a national wildlife disease surveillance system.

PREDICT-Mexico also organized and participated in three symposia at the National Autonomous University of Mexico (UNAM). The meetings were held on different campuses, and the number of attendees ranged from 30 to 100. These symposia focused on the effect of anthropogenic activities, climate change, and biodiversity loss on disease dynamics, as well as new strategies to monitor wildlife health. One of the symposia also highlighted the role of women in science.

A PhD candidate from UNAM training at CII, Columbia University in molecular methods, including deep sequencing.



Training Future Experts. PREDICT-Mexico supported the training of several Master's and PhD level students in disease ecology and evolution of disease at UNAM. Their research included pathogen discovery and the effect of species diversity and landscape change on the dynamics of infectious diseases.

One PhD student participated in a month-long exchange program to learn deep sequencing techniques at the Center for Infection and Immunity at Columbia University. The student's study was published in the Journal of General Virology and described the discovery of 12 novel coronaviruses and one known coronavirus in bats from Mexico (Anthony, Ojeda-Flores et al. 2013). At the time of publication, one of the discovered viruses had a 95% amino acid identity to the coronavirus causing the Middle Eastern respiratory syndrome (MERS) outbreak in the Middle East. This finding informed on targeted surveillance for coronaviruses in bats living in areas with cases of MERS.

Lasting Change. The PREDICT team's efforts in Mexico helped develop a One Health framework for emerging disease prevention and control on a national level. The team's Country Coordinator was invited to present to three Mexican governmental departments. It was the first time Mexico's health department (CONASA), agriculture department (SAGARPA), and health and natural resources department (SEMARNAT) came together to talk about connections between the health of wildlife and domestic animals. As a result of these efforts, a wildlife health position was established on the Technical Council of

PHOTO BY SIMON ANTHONY

Animal Health, and the PREDICT Country Coordinator was invited to serve a two-year term as the World Organisation for Animal Health (OIE) representative.

Other contributions to the future of One Health platforms were assured through PREDICT's significant contributions to higher education in Mexico. Our protocols were incorporated into the curriculum in the Disease Ecology and Conservation Medicine undergraduate course at the Veterinary School at UNAM. Furthermore, the PREDICT diagnostic laboratory protocols were implemented by other research faculty at the Veterinary School.

CAPACITY BUILDING

Training

Significant improvements in wildlife disease surveillance were made in Mexico as a result of PREDICT trainings on proper sample collection and management of data through the GAINS database. Thirty-two students and faculty members received training on the full set of protocols developed by PREDICT, including human and animal safety during wildlife capture, PPE, laboratory safety, sampling methodology, and zoonotic diseases. The team also trained 25 biologists in bat identification and more than 20 students on sampling of bats and rodents during a formal course at the Faculty of Medical and Veterinary Sciences, UNAM.

Following completion of the PREDICT project in Mexico, the local partners worked to ensure sustainability of in-country surveillance systems through efforts to implement a database similar to GAINS in Mexico that would integrate research from several Mexican universities. In addition, the partners developed a proposal with SEMARNAT to design a national database for epidemiological surveillance data for wildlife diseases.

Laboratory

PREDICT's laboratory diagnostic protocols for detection of viruses of public and animal health importance improved the ability to screen for important viral genera/families, such as coronaviruses, paramyxoviruses, flaviviruses, arenaviruses, and hantaviruses. These protocols were implemented in at least three university laboratories and one national public health laboratory. The Mexican government now has the capacity to utilize the PREDICT protocols along with the universal controls for viral family-level PCR screening. The university laboratories that implemented PREDICT diagnostic protocols trained more than ten undergraduate and graduate students.

SURVEILLANCE

Initial disease surveillance sites were chosen based on a gradient of likely virus spillover from animals into humans. EID hotspot data from Jones et al. 2008 was utilized to select sites in Mexico City, Chiapas, and in the Calakmul Biosphere Reserve representing areas with potentially high, medium, and low likelihood of viral spillover.

Because theoretical models suggest that land-use change is a primary driver of emerging infectious diseases (Loh et al. In Review), PREDICT Mexico added additional sites across a human disturbance

Members of the PREDICT Mexico team collecting samples from bats in a cave in Mexico.



PHOTO BY PREDICT MEXICO TEAM

gradient from pristine forests to more disturbed urbanized locations. Surveillance was then focused in two areas in southeast Mexico with very high biodiversity. For each of the two study areas, the field team chose sites along a landscape disturbance gradient, with sites located in pristine, fragmented, and disturbed locations within the same ecoregion in order to support the disease hotspots theory (Jones et al. 2008) and investigate the relationship between land-use change and host and viral diversity.

The pristine sites were located in the Lacandona and Calakmul Biosphere Reserves (in Campeche and Chiapas Mexico, respectively). Intermediately disturbed sites with partial deforestation (i.e. fragmented) were selected within 10 km of the reserves, and highly disturbed (i.e. disturbed) sites were selected in the same ecoregion. This approach was later further developed using a landscape disturbance index for the Deep Forest study in Brazil, Malaysia, and Uganda. The diversity and richness of bats was estimated by habitat gradient to describe the effect of habitat loss on the assembly of bat communities. The data presented corresponds to 12 nights of sampling effort by habitat type. The diversity of each habitat was calculated with the Shannon's index (H), and the richness (S) is the number of species in each habitat.

Surveillance was conducted at sites where there was potential for disease spillover from wildlife into people and one site where contact between wildlife and people was unlikely. The high-risk wildlife-human interfaces represented at the sites included wildlife in contact with park personnel/tourists or researchers, wildlife in contact with workers in extractive industries (e.g. logging, mining), and wildlife in or near human dwellings or agricultural fields (Figure 1; Table 1). Disease surveillance activities were focused on bats and rodents. A total of 2,028 animals were sampled (1,981 bats, 41 rodents, and 6 marsupials), representing 58 bat species and 11 rodent species (Figure 2).

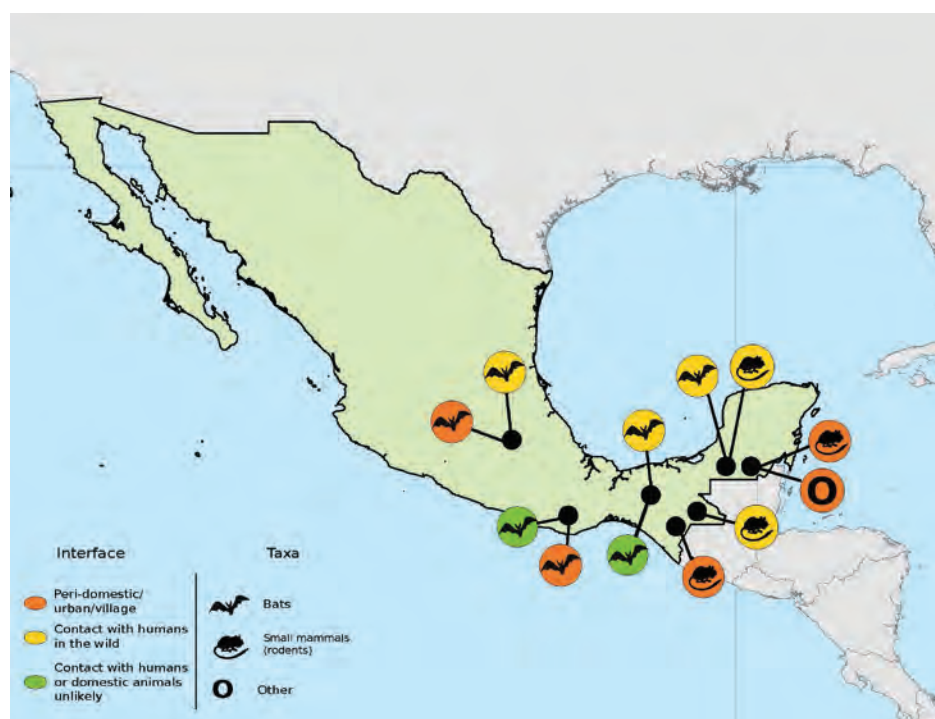


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

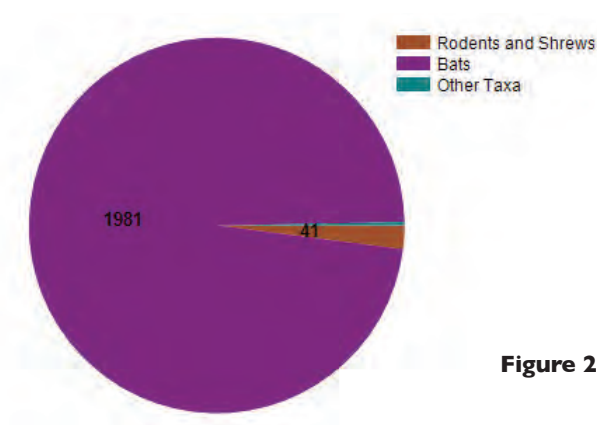


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	14	139	1
Ecotourism and recreational activities	0	270	0
Extractive industries	3	31	0
In or near human dwellings	22	631	5
Wildlife being studied	0	129	0
Protected areas	2	759	0
Pristine habitat	0	5	0
Zoos and sanctuaries	0	17	0
Total:	41	1981	6

VIRUS DISCOVERY AND CHARACTERIZATION IN BATS

Initially, consensus (genus/family level) PCR assays for 13 viral genera/families of health importance (coronaviruses, adenoviruses, herpesviruses, astroviruses, paramyxoviruses, filoviruses, bocaviruses, alphaviruses, flaviviruses, lyssaviruses, seadornaviruses, arenaviruses, and hantaviruses) were conducted on archived samples collected from 117 bats in the Mexican states of Chiapas, Oaxaca, and Tabasco. This work led to the discovery of novel viruses, including betaherpesviruses, gammaherpesviruses, coronaviruses, and astroviruses.

PREDICT also used unbiased high-throughput sequencing to uncover a highly diverse group of bat-derived viruses in bats from Africa, Mexico, and Central America that are related to hepaciviruses and pegiviruses within the family Flaviviridae. This testing identified seven new pegiviruses (*Flaviviridae*) in seven different species of bats (Quan et al. 2013). Evolutionary analyses indicate that all known hepaciviruses and pegiviruses, including those from humans and other primates, originate in bats (Quan et al. 2013). The biodiversity, phylogenetic divergence, and global distribution of the viruses suggest that bats are a natural reservoir for hepaciviruses and pegiviruses and further enhance our understanding of the evolutionary history of hepatitis C virus and the human GB viruses (Quan et al. 2013).

In addition, PREDICT-Mexico sampled bats for dengue virus in landscapes that had undergone anthropogenic changes and also pristine areas in the Calakmul (Campeche) and Montes Azules (Chiapas) Biosphere Reserves in southern Mexico. Six bats (4.1%) tested positive for dengue serotype 2: four bats in Calakmul (two *Glossophaga soricina* bats, one *Artibeus jamaicensis* bat, and one *A. lituratus* bat) and two bats in Montes Azules (both *A. lituratus*; Sotomayor-Bonilla et al. 2014). No effect of anthropogenic disturbance on the presence of dengue virus in bats was detected in this study; however, the species of bats exhibiting positive results are abundant and well-adapted to disturbed habitats in the Neotropics (Sotomayor-Bonilla et al. 2014). The role of bats in the ecology of dengue virus is unknown.

Investigation of Coronavirus Diversity in Bats

More in-depth research was conducted to investigate coronavirus diversity in 42 different species of bats sampled at Campeche, Chiapas, and Mexico City. Including those samples screened in the initial testing using consensus PCR, a total of 1,046 samples from 606 individuals were used in this assessment.

Broadly reactive consensus PCR revealed coronavirus (CoV) sequences in 5.3% (32/606) of bats. Sequence analyses indicated high phylogenetic diversity and the presence of 13 distinct clades at the nucleotide level. Nine of the viruses clustered with known alphacoronaviruses, and four clustered with betacoronaviruses. One of the alphacoronaviruses (Mex_Cov-6) was closely related to a virus identified previously in an *Eptesicus fuscus* bat sampled on the Appalachian Trail in Maryland, USA (Donaldson et al. 2010). As a result of this research, the known geographical range of this virus was extended to southeastern Mexico. In addition, 12 novel CoVs were discovered in the bats. Seven novel CoV genotypes were discovered in 17 bats sampled in Campeche (17/240), six novel CoV genotypes were found in nine bats sampled in Chiapas (9/332), and two novel CoV genotypes were detected in six bats captured in Mexico City (6/34). Four of these novel coronaviruses were found in more than one bat.

The PREDICT Mexico team with local community members.



PHOTO BY PREDICT MEXICO

Clustering of Coronaviruses Based on Phylogenetic Relatedness of the Host Species.

Phylogenetic analyses demonstrated clustering of coronaviruses based on phylogenetic relatedness (i.e. taxonomic family) of the host species (Figure 3; Anthony et al. 2013). Alphacoronaviruses detected in phyllostomid bats cluster together, as do alphacoronaviruses discovered in miniopterid bats. Virus isolates from the closely related Vespertilionidae and Molossidae families of bats also cluster together. Similar patterns of clustering associated with host relatedness were observed in the betacoronavirus sequences. Viruses detected in rhinolophid bats cluster together, as do viruses found in the closely related Vespertilionidae and Molossidae families and Mormoopidae and Phyllostomidae families (Anthony et al. 2013).

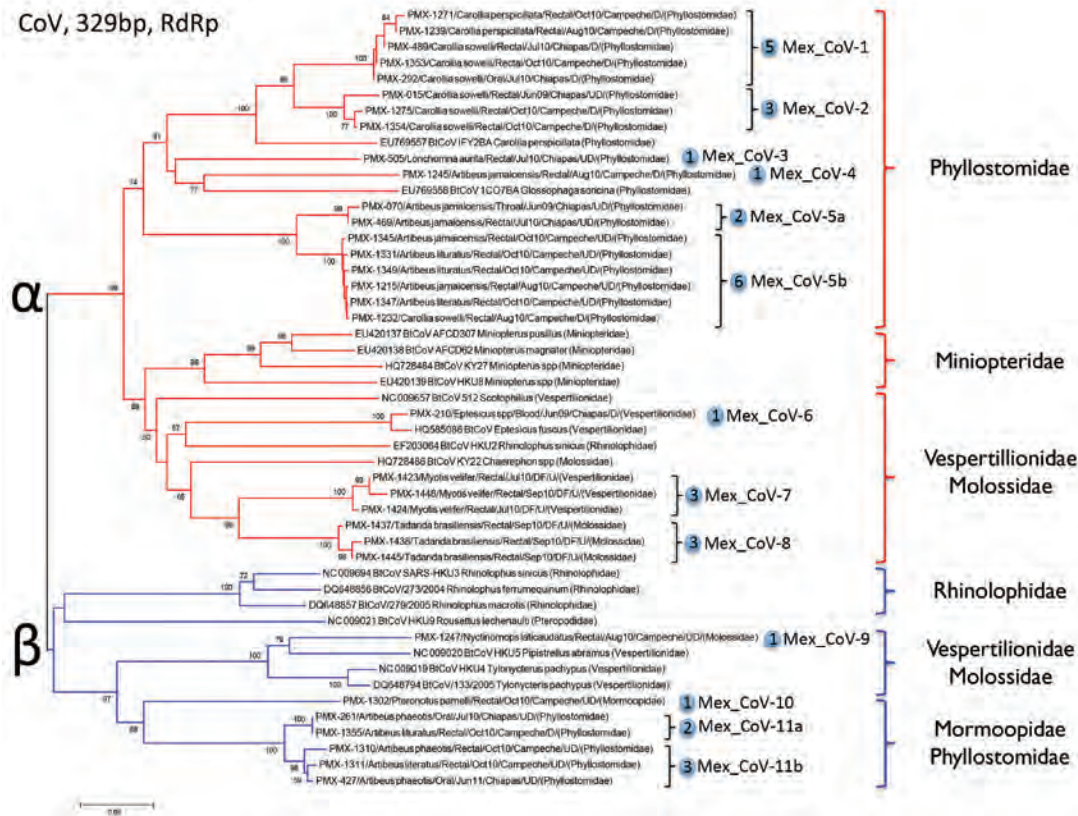


Figure 3. Maximum-likelihood tree of a 329 bp fragment of the RdRp from bat CoVs (red, a-CoVs; blue, b-CoVs). All 32 positive animals from this study are presented on the tree. CoVs identified in this study split into 13 clades. Each clade is indicated by a blue circle, and the total number of positive animals for each clade is indicated within. D =disturbed habitat; UD = undisturbed habitat; U = urban habitat. Bar, 0.05 nucleotide substitutions per site. From Anthony et al. 2013.

Only one coronavirus sequence (Mex_CoV-5b) in this study has been detected in two distinct (but related) genera of bats (Anthony et al. 2013). Such findings have been reported previously, albeit rarely (Tong et al. 2009; Osborne et al. 2011; Lau et al. 2012), and demonstrate that CoVs can infect individuals from different genera/suborders. This sequence originated from a bat (*Carollia sowelli*) captured in a disturbed habitat. Additional viral discovery efforts are needed in this region to investigate whether disturbed habitats increase the risk or opportunity for viruses to spillover into new species, as previously suggested (Cottontailusen et al. 2009; Keesing et al. 2010; Suzán et al. 2012).

Contributions to Targeted Surveillance of Bats in Saudi Arabia during MERS Outbreak.

PREDICT's discovery of a betacoronavirus closely related (96.5% amino acid identity) to the MERS-CoV in a *Nyctinomops laticaudatus* bat captured for this study (Anthony et al. 2013; Figure 4) directed targeted surveillance of bats in Saudi Arabia. These efforts led to the discovery of a MERS-CoV sequence in a Saudi bat that was identical (100% nucleotide identity) to the virus from a human case-patient (van Boheemen et al. 2012). These findings highlight the importance of virus discovery and the need for risk characterization to enhance our understanding of the potential risk posed to animal and human health by these novel viruses.

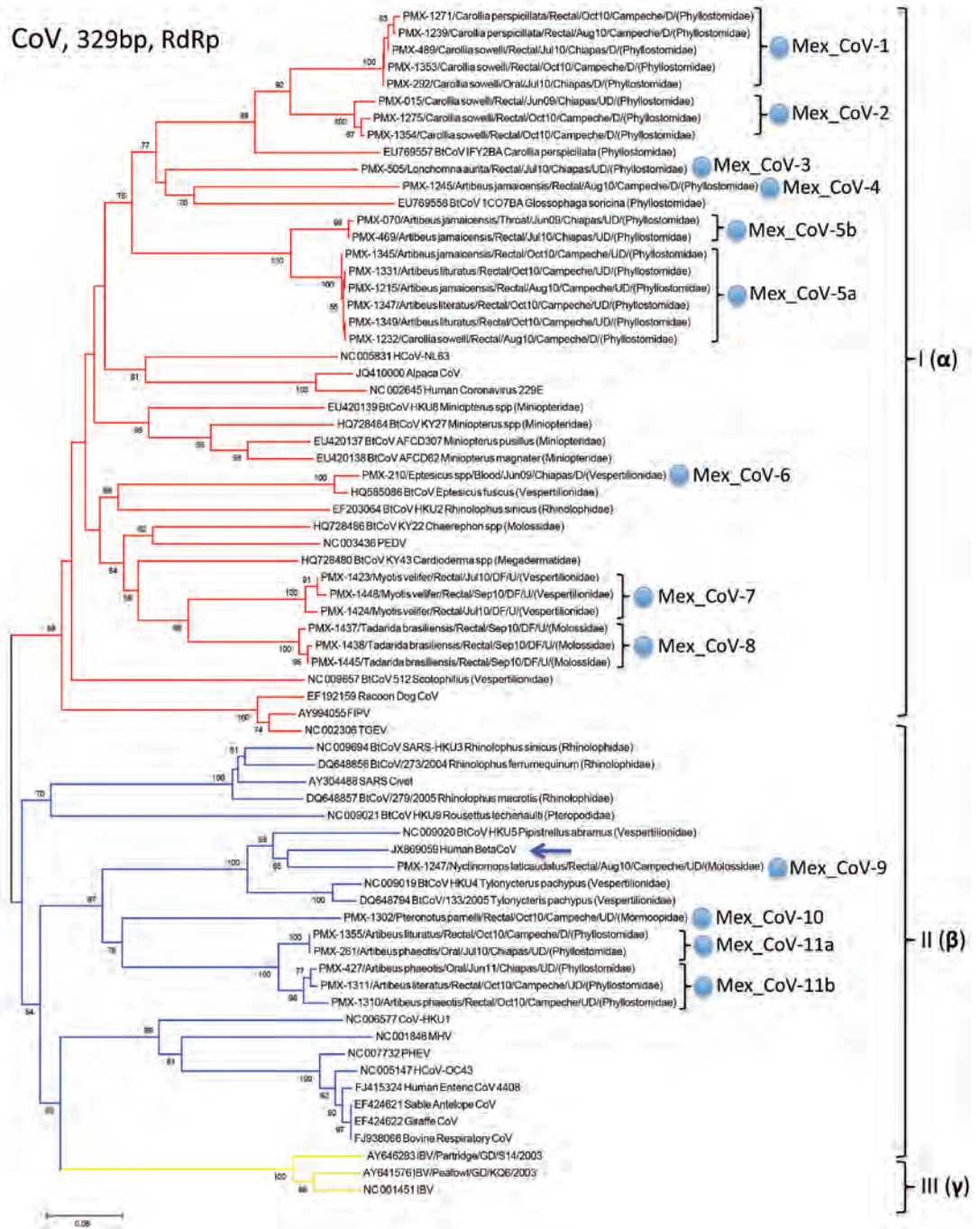


Figure 4. Maximum-likelihood tree of a 329 bp fragment of the RdRp from all CoVs (red, a-CoVs; blue, b-CoVs; yellow, c-CoVs). Viruses discovered in this study are indicated by blue circles. The 2012 human b-CoV is indicated by an arrow, and clusters most closely to PMX-1247/*Nyctinomops laticaudatus*. Bar, 0.05 nucleotide substitutions per site. From Anthony et al. 2013.

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PHOTOS BY NIGEL WALKER



Rapid urban and economic growth in Nepal have led to the expansion of urban infrastructure, increasing opportunities for contact among wildlife, domestic animals, and residents and tourists of the city.

NEPAL

In Kathmandu, Nepal, rapid urban growth spurred by conflict and a lack of urban planning stress the vulnerability of public health infrastructure, especially in areas of intensified new growth in former croplands, forest fragments, and along urban riverbanks. Riverfront property, often the most expensive urban real estate in many cities, has been in many parts of Kathmandu converted to informal settlement and agricultural production. Along the banks of the urban rivers and their tributaries, “*sukumbasi*” communities (landless squatters) now comprise some of the poorest areas of dwelling in the city. These *sukumbasi* communities demonstrate vast ethnic and caste diversity (Tanaka 2009) and may have shifted the city’s demographic composition and ecology, as subsistence agriculture and livestock husbandry within densely populated urban areas contributes to the livelihoods of the urban poor. Both people and animals living in these settlements have limited access to basic infrastructure (e.g. clean water and treatment of sewage), and these communities fall outside of health care systems.

In addition to the settlements, Kathmandu’s complex urban ecological tapestry is rich with other opportunities for human-wildlife contact, vividly illustrated by the monkey temple context at multiple locations throughout the city. Monkey temples, common in South Asia, where primates play a role in Hindu and Buddhist culture (Fuentes 2005), are typically home to large unmanaged macaque populations. Macaque species thrive in human-altered environments and often come in close contact with humans at these temple sites (Fuentes 2005). These temple sites may account for more human-primate contact than any other context (Jones-Engel 2005), as a diverse collective of workers, visitors, pilgrims, and international travelers visit the sites and their monkey inhabitants for worship, recreation, and tourism. These sites are critical interfaces for the transmission of zoonotic diseases, and previous studies have detected viruses with zoonotic potential in resident macaque populations (Jones-Engel 2006).

FOR A SUMMARY OF THIS SECTION GO TO WWW.NEPAL.PREDICT.GLOBAL

Further, economic growth and rural-to-urban migration in the Kathmandu Valley has led to the rapid expansion of urban infrastructure into formerly agricultural and forested areas, degrading wildlife habitat with unknown impacts on wildlife ecology, population dynamics, and type and frequency of human-animal contact. Kathmandu Valley is home to endemic colonies of giant fruit bats (*Pteropus giganteus*), known reservoirs for multiple zoonotic viruses and long-time residents of the Valley's forested areas, whose roosting sites are increasingly exposed to the pressures of urbanization. In part as a result of these pressures, bats at one particular site in Lazimpat, Kathmandu, roost in trees over the sidewalks of a major urban road. Bat excreta drops into pedestrian walkways, presenting a potential disease transmission risk to people and urban animal populations.

PREDICT-Nepal was initiated in 2011 (year three of PREDICT's five year timeline), in response to feedback from the External Advisory Panel to explore the risk for disease emergence at human-animal interfaces in urban ecosystems. In Nepal, PREDICT worked to strengthen local capacity to characterize zoonotic disease risks and improve understanding of disease transmission, to lay the groundwork for disease surveillance systems for wildlife, and to assist the country in preparations for enhanced response to zoonotic disease outbreaks. Through PREDICT-Nepal's implementing partner at the Center for Molecular Dynamics, Nepal (CMDN), research was conducted to characterize the viral diversity among taxonomic groups considered high-risk for disease emergence in Kathmandu (bats, nonhuman primates, and rodents), focusing on areas considered high-risk for human-animal contact like the *sukumbasi* informal settlements, highly-trafficked urban centers, and monkey temple complexes. PREDICT-Nepal systematically sampled urban wildlife at these interfaces and collected specimens from bats, rodents, and nonhuman primates to assess viral diversity and the potential public health significance. Findings from the project are helping to improve recommendations for disease surveillance and prevention in Nepal.

PARTNERS

PREDICT partners in Nepal included the Center for Molecular Dynamics Nepal (CMDN), UC Davis (UCD), and EcoHealth Alliance (EHA), working together to strengthen partnerships in-country and to establish field sites targeting areas with high levels of contact among people and wildlife, focusing on taxa that have the greatest contact with humans such as bats, rodents, and nonhuman primates.

PREDICT built upon existing CMDN relationships with in-country governmental, public, and private institutions and identified new partnerships with key agencies, ministries, and non-governmental organizations.

Collaborators included:

- The Department of National Parks and Wildlife Conservation
- Ministry of Agriculture Development, Department of Livestock Services and Central Veterinary Laboratory
- Ministry of Health and Population, Department of Epidemiology and Disease Control
- Ministry of Health and Population, Department of Health Services National Public Health Laboratory

PREDICT Nepal field team discusses capturing rodents with community in Kathmandu.



PHOTO BY NIGEL WALKER

- Nepal Health Research Council
- Patan Academy of Health Sciences
- The One Health Alliance of South Asia
- Tribhuvan University
- Tribhuvan University Institute of Agriculture and Animal Science
- USAID

MAJOR ACHIEVEMENTS

- Supported the organization of Nepal's first One Health workshop in collaboration with the One Health Alliance South Asia (OHASA) Nepal Chapter to improve coordination among government, NGO, academic, and private sector stakeholders and held meetings with OHASA to share findings and discuss future collaborations leading to the formation of the One Health Association of Nepal (OHAN; see Success Stories for more information).
- Established culturally appropriate non-invasive surveillance techniques for wildlife disease surveillance (see Success Stories for more information).
- Prioritized high-risk disease transmission interfaces and locations for surveillance activities using innovative interface characterization methods through a combination of local knowledge, geographic information systems, scientific literature, feedback from ministry partners, direct observation, and unstructured interviews during field visits.
- Rapidly deployed surveillance in urban areas of Kathmandu and completed animal sampling and pathogen testing targets within an 18-month timeframe during which the PREDICT project was active in Nepal.
 - Trained 11 individuals on PREDICT animal capture, handling, and sampling protocols, and a total of 16 individuals in field surveillance techniques.
 - Selected 11 sites following intensive site characterization surveys of human-animal interfaces in the Kathmandu Valley for surveillance activities.
 - Sampled a total of 768 animals (109 bats, 411 rodents and shrews, and 248 nonhuman primates).
- Improved the infrastructure, capacity, and knowledge for viral detection in wildlife in Nepal. In less than a year, trained lab technicians at Center for Molecular Dynamics-Nepal, in close coordination with the University of California Davis laboratory team, and tested wildlife specimens for 10 viral families.

SUCCESS STORIES

One Health Association of Nepal

Nepal's first One Health Workshop was organized in 2012 bringing government and non-government stakeholders from animal, human, and environmental health together to highlight the importance of the One Health concept as a possible means to foster cross-sector collaboration. Regional experts conducted a workshop to assess the current status of

cross communication and collaborations between various sectors and developed recommendations to initiate One Health focused efforts in Nepal. This event was co-sponsored by PREDICT and WHO/Nepal and was organized by the National Zoonoses and Food Hygiene Research Centre.

As a result of the workshop, stakeholders created the One Health Association of Nepal (OHAN), which was later registered as a non-profit organization with the Government of Nepal. Executive members of OHAN, including PREDICT-Nepal's principal investigator, participated in the South Asia Regional Workshop for One Health held in Kathmandu, Nepal.

Following these workshops, there was greater realization from all sectors on the relevance and importance of a One Health approach in the implementation of disease surveillance, detection, prevention, and control programs. Through OHAN, Nepal developed a platform to launch initiatives connecting all health-related systems and stakeholders (animal, human, and environment) and to provide communication channels for information flow and enhanced inter-agency and transdisciplinary coordination.

Culturally Appropriate Surveillance Techniques

In Nepal, culture and religious tradition influence selection of sampling techniques for wildlife disease surveillance. Monkeys are religiously important to Hindu communities in Nepal as they represent Hanuman, a revered deity. Because invasive sampling of monkeys is restricted in Nepal, PREDICT implemented a novel non-invasive technique to collect macaque oral samples at monkey temples in Kathmandu, in partnership with UC Davis in the USA and the Mountain Gorilla Veterinary Program in Uganda.

The approach was developed and refined with laboratory colony rhesus macaques at the California National Primate Research Center, UC Davis, where optimization of sample collection, virus recovery, and virus detection was performed. In short, ropes dipped in fruit jam or banana baby food were provided to macaques to chew on and then retrieved by an attached string when discarded. Oral swab samples were also collected from the animals for comparison of virus recovery between the paired samples collected through the non-invasive and invasive methods. Following successful optimization of the technique in controlled settings and recovery of DNA and RNA viruses from the non-invasive ropes, the approach was field-tested with free-ranging arboreal and ground-dwelling non-human primates in Uganda and Nepal to evaluate both behavioral acceptance of the ropes along with recoverable sample quality in a field setting.

In Nepal, the PREDICT surveillance team worked with UC Davis veterinarians to pilot the approach among macaque communities living at three heavily trafficked temple sites, including two UNESCO world heritage sites (the Buddhist stupa Soyumbu and the Hindu temple Pashupatinath), where macaques frequently touch, scratch, and bite visitors and workers as they scavenge for food. The team evaluated multiple ways for dental rope introduction and retrieval before settling on observation and placement of a rope on the ground for macaques, then observing and following the animals until they lost interest and the swab could be safely retrieved



PHOTO BY MANISHA BISTA

Workshop participants at the One Health Alliance of Nepal.

PHOTO BY THE PREDICT NEPAL TEAM



PHOTO BY NIGEL WALKER



Non-invasive collection of macaque saliva at temples in Kathmandu.

and placed in a cooler for transport. Dental ropes were centrifuged by PREDICT lab technicians and then processed and tested for five viral families. Through this approach, PREDICT successfully detected RNA virus in multiple animals (Simian Foamy Virus), confirming that the non-invasive saliva collection technique using ropes is a valid sampling method for disease surveillance in difficult to capture animal populations where invasive techniques and immobilization may not be feasible (Smiley Evans et al. 2014).

CAPACITY BUILDING

Infrastructure Development

PREDICT improved wildlife surveillance capacity in Nepal through provision of animal capture and sampling supplies and cold chain equipment for sample storage. In addition, PREDICT enhanced facilities for molecular diagnostics to improve Nepal's capacity for viral detection through the procurement of ultra-low temperature freezers and liquid nitrogen cold chain, sample storage materials, viral detection supplies including the universal control material for consensus based (genus/family level) PCR, and information management protocols through the GAINS system.

Training

Surveillance. PREDICT-Nepal trained 21 individuals in biosafety-PPE; safe animal capture and handling; laboratory safety and responsibility; rodent, primate, and bat sample collection; data recording and information management through the GAINS platform; cold chain maintenance; and safe sample shipment. In addition, intensive on-the-job training was conducted with the surveillance team on risk characterization and site assessments to refine surveillance planning and targets to best align with global and country-based objectives. During the human-animal interaction survey (see below), four field researchers and enumerators were trained on the use of a mobile application for data collection, improving data quality, and enabling feedback from the collaborative research team. Specialized training was conducted with six individuals in non-invasive saliva collection from macaques (see Success Stories above). Finally, in collaboration with partners at EcoHealth Alliance, the PREDICT Bangladesh Country Coordinator visited Nepal to conduct field-based training with eight individuals in habitat identification, roost counts, and

non-invasive sampling of urban fruit bat colonies in areas of Kathmandu where mist netting and other capture techniques were determined inappropriate or overly risky, based on the location of roosting sites in trees over heavily trafficked sidewalks, roads, and public buildings.

Pathogen Testing. PREDICT enhanced Nepal's existing molecular diagnostic capacity by training 17 individuals in laboratory safety and six individuals in nucleic acid extraction and consensus-based (genus/family level) PCR for 10 viral families (arenaviruses, bunyaviruses, coronaviruses, filoviruses, hantaviruses, henipaviruses, influenza viruses, paramyxoviruses, retroviruses, and rhabdoviruses), equipping the project laboratory in Nepal with the capability to screen for a broad range of potential viral pandemic threats in order to support outbreak investigations for diseases of unknown origin, and networking the laboratory with global centers of excellence in advanced molecular diagnostics (e.g. deep sequencing).

SURVEILLANCE

PREDICT-Nepal focused surveillance and animal sampling efforts on urban wildlife in Kathmandu at high-risk human-animal contact interfaces filling a gap in PREDICT's global surveillance approach (Figure 1 and Table 1). In Kathmandu, PREDICT targeted high-risk urban interfaces (areas with close human and animal contact around homes and dwellings) at seven locations for rodent and shrew surveillance in informal settlements along the Manahara, Bagmati, and Bishnumati Rivers and their tributaries. Rodents and shrews were sampled in and around human residences and dwellings and in fields and markets in urban gardens and on the urban periphery where animals damage crops and marketable produce.

Sampling was also conducted at three high-risk peri-domestic and ecotourism interfaces for nonhuman primates using non-invasive techniques. These sites are religious and tourist attractions where people are frequently touched, scratched, and bitten by macaques while feeding habituated animals. Fruit bats (*Pteropus giganteus*) were sampled non-invasively along a main thoroughfare where the bats co-roost with birds in trees lining a main urban road adjacent to a large tourist attraction, and where animal excreta fall onto heavily trafficked sidewalks. Additionally, surveillance was conducted at select sites in wet and dry seasons to assess seasonal variation in viral shedding (including repeated sampling of rodent and shrew populations at four of the unplanned settlement sites and macaques at one of the religious sites).

PREDICT-Nepal sampled a total of 768 animals (411 rodents and shrews, 248 nonhuman primates, and 109 bats; Figure 2). Specimens were prioritized for testing based on probable disease transmission pathways associated with each taxonomic group and interface, and specimens were tested for the five priority viral families for each taxonomic group in Nepal and an additional nine viral families at UC Davis. Products for sequencing were forwarded for confirmatory testing, cloning, and sequencing at UC Davis.

Mobile sampling tent and successful isolation of sampling site in the busy squatter area or "settlement".



PHOTOS BY PREDICT NEPAL TEAM



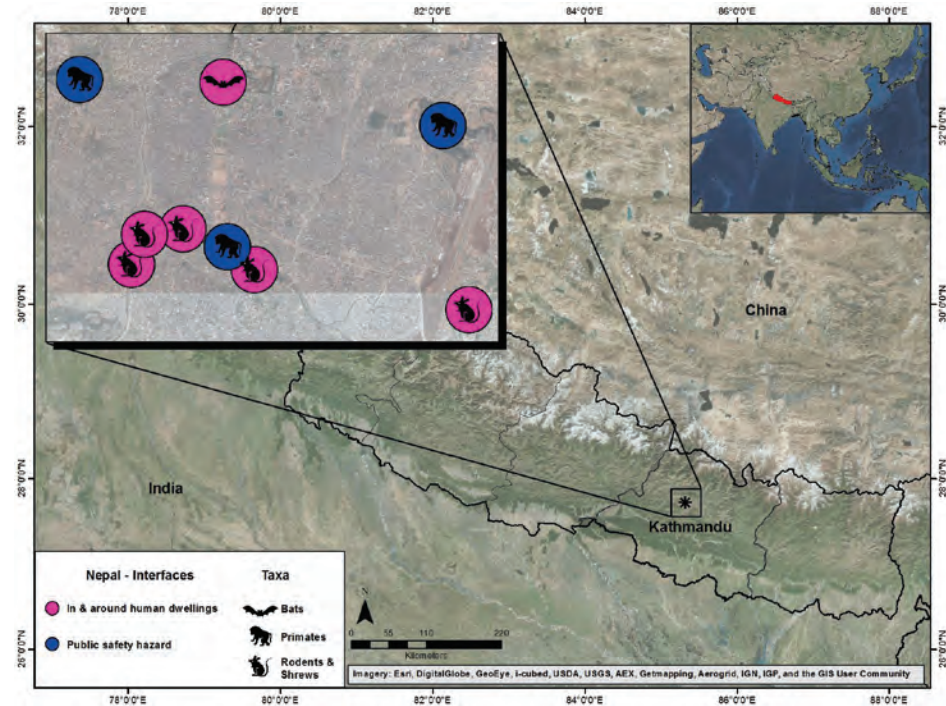


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

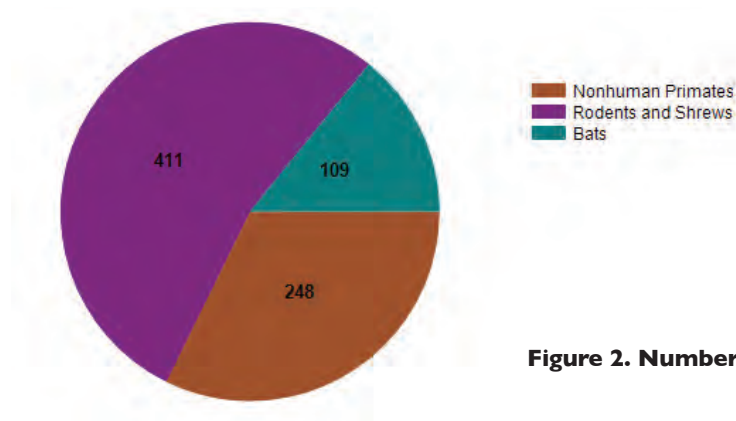


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats
In or near human dwellings	248	411	109
Total:	248	411	109

PREDICT-Nepal conducted a survey to assess human-animal contact and disease transmission risk at nine animal surveillance sites: six sites where rodents and shrews were sampled and three sites where macaques were sampled. The survey was combined with the pilot testing of an Android-platform application developed as a data collection tool to enable real-time data capture and sharing with the collaborative research team in Kathmandu and the US. Survey data were combined with test result data from animals sampled at the same sites to characterize potential disease risk and transmission pathways from animals to humans, with findings improving our understanding of the type and frequency of human-animal contact to help identify disease prevention recommendations.

PREDICT-Nepal also participated in the Local Medial Surveillance (LMS) study (see Information Management section and Schwind et al. 2014 for more information), conducting surveillance of local digital and print media sources for news of disease and health risk events not integrated into current digital disease detection systems via HealthMap, in collaboration with seven other PREDICT countries.

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PHOTO BY DIBESH KAARMACHARYA



The PREDICT Nepal team administers a human-animal contact survey in Kathmandu and pilot tests a mobile application for data collection, allowing the international research team real-time visualization of data for feedback and project optimization.



PHOTOS BY PATRICIA MENDOZA

Despite efforts by officials to develop policy prohibiting wildlife trade, this activity is still rampant throughout Peru and especially prevalent in wet markets.

PERU

On the list of the ten most biologically diverse countries in the world, Peru boasts a rich variety of ecosystems, species, genetic resources, and cultures (Mittermeier et al. 1997). Cutting through the country from North to South, the Andes mountain range determines Peru's variety of habitats and high degree of biodiversity heterogeneity (Brack and Mediola 2000). However, despite the natural treasures present, Peruvian biological wealth is ignored and overharvested. Rapid economic and demographic growth occurred in recent years, driving major ecological changes that are especially noticeable in the Amazon forest. The main activities leading to biodiversity loss are deforestation, agricultural expansion, and extractive industries, which have facilitated illegal logging and wildlife trade. These anthropogenic impacts are becoming more apparent and threatening, and there are clear examples in Peru of a link between these impacts and the emergence of infectious diseases in people.

Mining is exerting a powerful force for change in the Peruvian Amazon. Between 1999 and 2012, the geographical area impacted by mining expanded by 400% in Madre de Dios, one of the most biodiverse regions of the world (Asner et al. 2013). As a result of the global financial crisis in 2008 and the high international price of gold, a "gold rush" exponentially affected Amazon territory, with losses in forested areas increasing from 2,166 to 6,145 hectares per year (Asner et al. 2013). Deforestation, driven by human economic activities, is also prevalent in much of the Peruvian territory. For example, land conversion for oil palm crops, fueled by the biodiesel industry, is increasing in the northeastern Peruvian Amazon (Dammert et al. 2012).

These changes have a direct impact on biodiversity, causing loss of species and alterations in the ecological balance of ecosystems. With over 500 native species of mammals, Peru is the third most diverse country in the Americas and the fifth in the world (Pacheco et al. 2009).

FOR A SUMMARY OF THIS SECTION GO TO WWW.PERU.PREDICT.GLOBAL

Mammals in Peru are largely dominated by bats (165 species) and rodents (162 species), representing 64% of all species. An equal diversity of pathogenic microorganisms related to these mammalian hosts, many of them potentially zoonotic, can be expected. Disease outbreaks stemming from bat and rodent reservoirs heavily burden the health and welfare of human populations in remote areas. The expansion of sugar cane crops and associated migrant labor caused changes in the ecology of rodents and vectors in Peru's northwest area, sparking outbreaks of plague in 2010 (Pachas et al. 2010). Similarly, sylvatic rabies caused by vampire bats dramatically affects indigenous communities with poor access to health services throughout the Peruvian Amazon (Condori et al. 2013).

Furthermore, as a highly diverse country, Peru is also an active supplier for the international market of wild pets, both by legal and illegal means (Ríos et al., 2008). Despite international agreements and ongoing efforts at the national and regional levels for the development of policies that prohibit wildlife trade, this activity is still rampant throughout Peru and especially prevalent in wet markets (Gastañaga et al., 2010; Ortiz, 2010; Rios et al. 2008).

The aforementioned anthropogenic pressures increase Peru's vulnerability to the emergence of zoonotic diseases of wildlife origin. Strengthening government capacities, particularly for disease surveillance strategies that allow for predicting disease occurrence and identifying high-risk interfaces for zoonotic transmission, is necessary. To this end, the PREDICT project was implemented in Peru from 2010 to 2014 as a collaborative effort between the Wildlife Conservation Society (WCS), the Peruvian Veterinary Service (SENASA), and the Peruvian Institute of Health (INS). PREDICT's strategy for improved detection and prevention of zoonotic diseases takes a One Health approach, recognizing that human activities and the health of humans, animals, and the environment are linked. Wildlife disease surveillance in Peru was focused on the detection of pathogens that can spread through subsistence hunting practices or be introduced to urban environments through the wildlife trade at wet markets. The approach was expanded to include active surveillance for novel pathogens in bats and rodents in and around human dwellings and in wildlife targeted for disease control and zoonotic outbreak investigations by government partners.

PARTNERS

The PREDICT Peru team was a collaborative effort between the Wildlife Conservation Society (WCS), the Peruvian Institute of Health (INS), USAID, and the Peruvian Animal Health Service (SENASA).

Other local partners included:

- School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos
- US Naval Medical Research Unit Six (NAMRU-6)
- General Directorate of Epidemiology, Ministry of Health
- General Directorate of Forestry and Wildlife, Ministry of Agriculture

PREDICT team collects samples from an anesthetized primate confiscated from the illegal wildlife trade and placed at a rescue center.

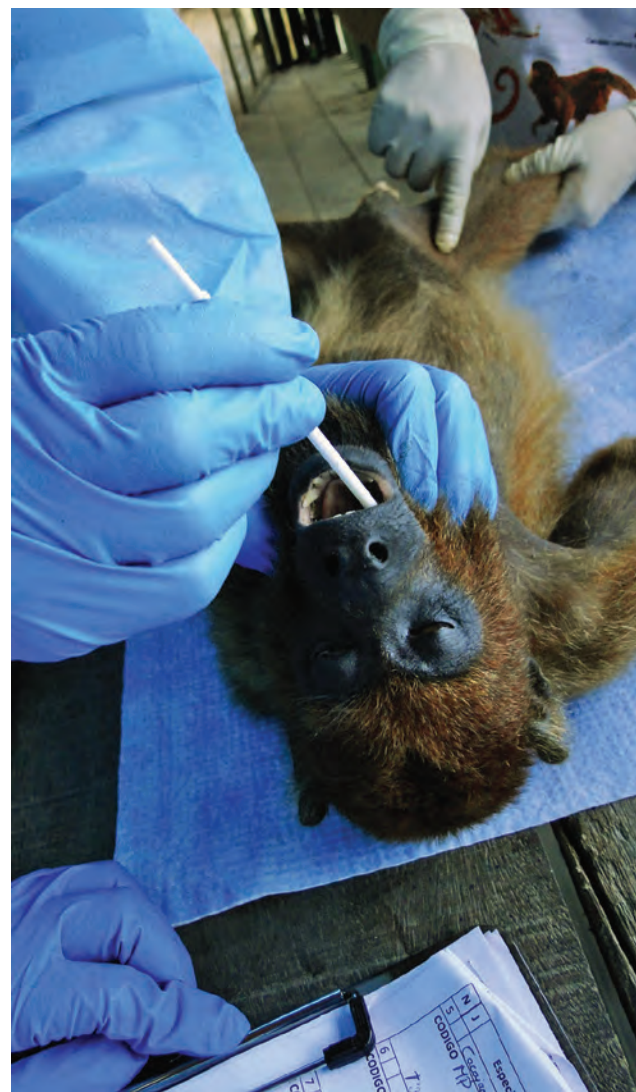


PHOTO BY PATRICIA MENDOZA

- Regional governments of Loreto and Ucayali
- Universidad Nacional de Tumbes
- Zoo Parque Natural de Pucallpa, Ucayali
- Varillal Temporary Custody Center, Loreto
- Ikama Peru Rescue and Rehabilitation center, Amazonas
- Taricaya Rescue Center, Madre de Dios
- Amazon Shelter Rehabilitation and Rescue Center, Madre de Dios

MAJOR ACHIEVEMENTS

- Enhanced capacity and improved cooperation among agencies, which led to implementation of effective health surveillance strategies and policy on a national scale. PREDICT's strategies for surveillance and laboratory procedures were implemented by INS nationwide (see Success Stories for more information).
- Enhanced reporting of domestic animal die-offs at markets to SENASA through a network of collaborators trained through PREDICT.
- Improved networks for disease communication and response among key government agencies, scientific institutions, and people at risk and formalized agreements with partners to ensure sustainability of the PREDICT One Health approach for wildlife disease surveillance and research.
- Developed capacity to conduct PCR using PREDICT protocols on human and animal samples in INS laboratories and expanded the suite of pathogens for screening during outbreak investigations.
- In cooperation with the Peruvian Directorate of Forestry and Wildlife (DGFFS), published guidelines for and assisted with management and sampling of wildlife confiscated from the illegal wildlife trade.
- Engaged stakeholders in the national wildlife disease surveillance system (VEAS) managed by SENASA.
- Highlighted the importance of wildlife disease surveillance at human-animal interfaces with high-risk for disease transmission by detecting a broad range of zoonotic pathogens.

SUCCESS STORIES

Enhanced Capacity and Improved Cooperation with and among Governmental Agencies

PREDICT significantly enhanced local capacity to detect and prevent zoonotic diseases of wildlife origin in Peru. A key indicator of success is the high level of trust and collaborative goodwill generated with the central government (i.e. Ministry of Health and SENASA). This enhanced cooperation was illustrated by the degree of engagement and participation of leaders and decision-makers in workshops for strengthening government capacity for wildlife disease surveillance, building upon SENASA's existing wildlife disease monitoring network. Surveillance

of wildlife-borne pathogens at wildlife-human interfaces was a high priority for the Peruvian government. As a result, PREDICT's strategies for improving surveillance and diagnostic laboratory capacities in Peru were adopted as a joint initiative by INS in 2011 and formally incorporated into the INS Strategic Operational Plan in 2013. Accordingly, the following strategies were adopted and implemented on a national scale:

- Pathogen detection at wildlife-human interfaces with high-risk for disease transmission (wildlife for sale in wet markets, wildlife in zoos and rescue centers, and subsistence hunting of wildlife);
- Viral pathogen surveillance in peri-urban wild animal reservoirs (bats and rodents); and
- Capacity building at the INS laboratory for consensus (virus genus/family level) PCR.



PREDICT increased capacity for wildlife sampling among government officers. The monkey pictured here was naturally deceased and provided by the local zoo for training purposes.

PREDICT Staff Member Awarded the 2014 Pedro N. Acha Award for Excellence

Micaela De La Puente from the PREDICT Peru team was awarded the 2014 Pedro N. Acha Award for Excellence in Veterinary Public Health on behalf of her thesis “Zoonotic Enteric Bacteria in Captive Neotropical Primates from Perú” (“Bacterias Entéricas con Potencial Zoonótico en Primates Neotropicales Mantenidos en Cautiverio, Perú”) in recognition of her dedication to the highest standards of study and professionalism in the veterinary health field in her early career. She received the award in a public ceremony in Washington, D.C. on September 29, 2014 (<http://www.pahofoundation.org/en/news/411-winners-of-awards-for-excellence-in-public-health-announced-awardees-hail-from-argentina-bolivia-mexico-peru-and-the-united-states.html>). This award is one of six Awards for Excellence in Inter-American Public Health, given by the Pan American Health Organization (PAHO) and the PAHO Foundation since 1975, recognizing the excellence in various areas of health that are vital to Americas. Dr. De La Puente's undergraduate thesis demonstrated the presence of zoonotic bacteria in 19% of neotropical monkeys in wetmarkets, wildlife refuges, and zoos in Peru, illustrating the potential health threat that monkeys removed from their environments and placed in poor housing conditions can pose to humans.

CAPACITY BUILDING

Training

In order to develop a sustainable surveillance system, PREDICT provided training in wildlife sampling and surveillance methods to 346 people in Peru, including field staff; veterinarians; laboratory technicians; biologists; indigenous community residents; and personnel from the public health, veterinary, and other government sectors. Standardized methods and innovative



Regional coordinator for PREDICT in South America, Dr. Uhart (front left), trains government officers in wildlife necropsy and sample collection.

tools were developed to ensure proper collection of information and samples at high-risk human-wildlife interfaces. Training topics included biosafety, animal capture, proper data and sample collection, cold chain, sample packing and shipment, and laboratory safety. Biosafety was emphasized to reduce occupational hazards to field and laboratory personnel. Questionnaires were completed at the end of each training session to ascertain knowledge transfer.

Diagnostic Laboratory Capacity

Diagnostic capacity was established at INS laboratories for screening bat, primate, and rodent samples for 12 viral genera/families of pandemic potential using broadly reactive consensus PCR assays (alphaviruses, arenaviruses, bunyaviruses, henipaviruses, coronaviruses, filoviruses, flaviviruses, hantaviruses, orthomyxoviruses (influenza), paramyxoviruses, retroviruses, and rhabdoviruses).

SURVEILLANCE

Surveillance was conducted in priority wildlife taxonomic groups (i.e. bats, nonhuman primates, and rodents) at critical wildlife-human interfaces with high risk for disease transmission in Peru (Figure 1 and Table 1) with a focus on wildlife hunted for subsistence by indigenous communities, wildlife for sale in wet markets or confiscated in the wildlife trade,

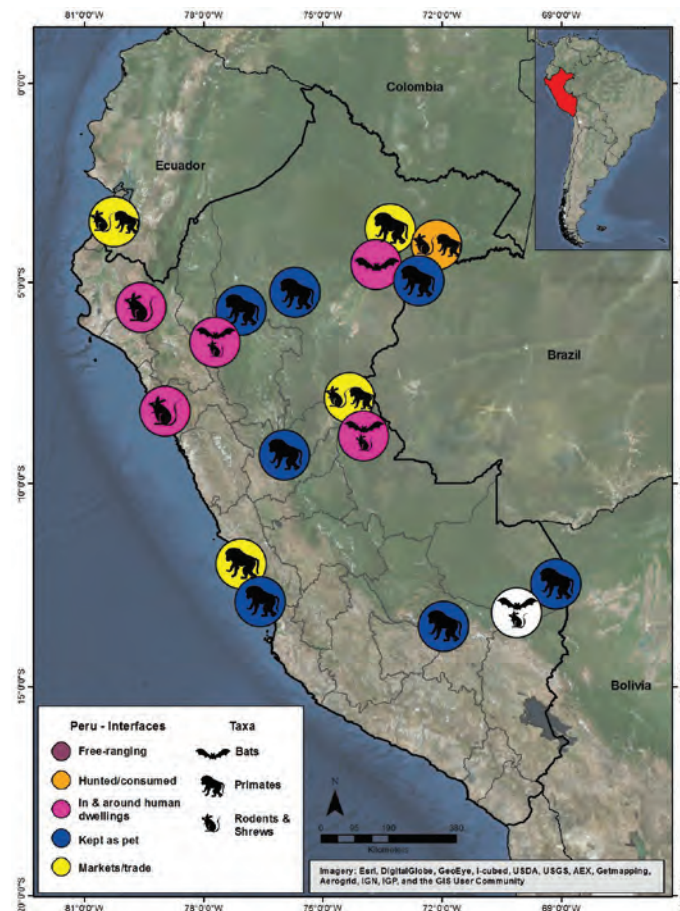


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

wildlife in captive settings (sanctuaries, rehabilitation centers, and zoos), and wildlife in peri-domestic settings (i.e. in and around human dwellings or fields). In addition, PREDICT conducted surveillance in wildlife during disease outbreaks in people. Free-ranging wild animals were also sampled in remote areas without human disturbance for baseline comparison.

A total of 80 sampling events were conducted in Peru, with 1,433 rodents, 535 nonhuman primates, 170 bats, and 667 animals from other taxa sampled for pathogen screening (Figure 2). Collection of blood samples on filter paper (e.g. dried blood spot cards) and in sample preservation solutions were employed to collect and preserve samples in remote locations where maintenance of a cold chain in the field was not feasible.

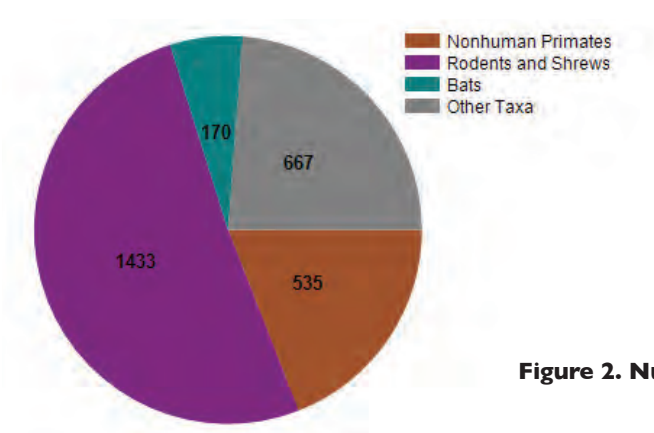


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
In and near human dwellings	28	1226	12	28
Hunted wildlife	83	69	0	175
Markets	97	103	0	329
Wildlife trade	110	3	0	107
Zoos and sanctuaries	216	14	0	24
Pristine habitat	1	18	158	4
Total:	535	1433	170	667

In order to ensure sustainability, PREDICT facilitated communications and forums to streamline national strategies for wildlife disease surveillance. Partnerships were formalized with seven institutions including ministries, laboratories, academia, NGOs, and civil organizations. PREDICT provided guidance and assistance to government agencies in more than 12 surveillance efforts; notable examples include bat and rodent sampling campaigns conducted during disease control programs and a number of disease outbreak investigations (e.g. vampire-bat rabies in Loreto, leptospirosis in Ucayali, and plague in Cajamarca). Key counterparts

(market vendors and staff from parks, zoos, and rescue centers) were committed to reporting animal disease events at wildlife-human interfaces and were instrumental in establishing a wildlife disease surveillance network. Surveillance in free-ranging bats and rodents was conducted in collaboration with scientific institutions, including the Natural History Museum of Arequipa and Universidad Mayor de San Marcos.

OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT also improved capacity for outbreak response in Peru by providing training and assistance to government agencies during field investigations. PREDICT aided the INS national task force in epidemiological surveillance in Peru during three zoonotic disease outbreaks associated with wildlife. Response efforts were focused on active disease surveillance in urban and peri-urban animal reservoirs and training of field staff from public agencies, universities, and NGOs on wildlife sampling and disease reporting.

Specifically, public health and veterinary agencies, including INS, the Regional Directorate of Health of Loreto, the Regional Directorate of Environmental Health of Ucayali, and SENASA reached out to PREDICT for assistance with wildlife sample collection and laboratory diagnostics during the following outbreaks:

- Pneumonic plague in Cajamarca and La Libertad in northwestern Peru from 2010 to 2013;
- Hantavirus pulmonary syndrome in Iquitos in the Loreto Region in 2011 (second historic record in country); and
- Leptospirosis in Pucallpa in the Ucayali Region in 2011.

Dr. Zariquiey processes samples in the field prior to shipment to the laboratory for diagnostic testing.



PHOTO BY PATRICIA MENDOZA

INVESTIGATION OF ZOOONOTIC PATHOGENS IN HUNTED WILDLIFE

PREDICT contributed to a number of studies investigating the presence of zoonotic pathogens in hunted wildlife in Peru. Arboviruses cause significant illness and death in South America, yet sylvatic cycles and the role of wildlife in the ecology of these viruses is still poorly understood. Outbreaks of disease in wildlife preceding human cases of yellow fever have been recognized in Brazil and Panama, suggesting linked transmission of this virus between wildlife and human populations. PREDICT aided in assessing exposure (i.e. serologic antibody titers) of hunted wildlife and domestic animals to flaviviruses and alphaviruses in a wildland-rural interface in the northeastern Peruvian Amazon where humans, animals, and vectors have close interaction. Results indicated that ungulates had the highest exposure to flaviviruses and alphaviruses followed by rodents and edentates (i.e. anteaters and sloths; Mayor et al. 2013). Specifically, ungulates had high antibody titers against St. Louis Encephalitis Virus (SLEV) and yellow fever virus (YFV). Rodents had high titers against Venezuela Equine Encephalitis Virus and SLEV, and edentates expressed high antibody titers against YFV and SLEV. In addition, animals sampled at the relatively disturbed site (i.e. high deforestation and encroachment of land for agricultural use and cattle ranching) had higher exposure

compared to the more pristine site, suggesting a higher risk of arbovirus infection in areas undergoing land-use change (Mayor et al. 2013).

PREDICT also assisted with research that explored the occurrence of *Toxoplasma gondii* exposure (a zoonotic parasite) among hunted peccaries, brocket deer, and lowland tapir in the Peruvian Amazon as a model for pathogen sharing. For this study, blood spot samples were obtained from animals hunted in the area surrounding the community of Nueva Esperanza where human cases of ocular toxoplasmosis had been documented. Evidence of exposure was common in the hunted ungulates (17-40%), suggesting a potential source of *T. gondii* infection in this community (Aston et al. 2014). Seroprevalence was lower in this study relative to other surveys of wild ungulates in less remote locations in the Amazon with domestic and feral cats.

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In RoC, rural communities rely heavily upon wildlife hunting for food and for income. Bushmeat is consumed by local subsistence hunters and is also sold in markets for wider consumption.

PHOTOS BY GLOBAL VIRAL CAMEROON (LEFT) AND BY GLOBAL VIRAL (RIGHT)

REPUBLIC OF CONGO

The Republic of Congo (RoC) is home to a number of key wildlife species such as chimpanzees, gorillas, and forest elephants. Important conservation zones protect large tracts of forest, which make up 66% of the area of the 341,500 km² of the country (World Bank 2014) and are especially dominant in the north. These remote forests provide habitat for the highest known densities of lowland gorillas, as well as other endangered high profile species. RoC encompasses a diversity of landscapes, including the basin of the Congo River.

In RoC, 36% of the population of 4.3 million people lives in rural areas and 31% of the land area is agricultural (World Bank 2014). Rural communities rely heavily upon hunting for food and for income. Bushmeat is consumed by subsistence hunters and also sold in markets. Hunting can expose individuals to infections from wildlife through bites and scratches, direct contact with animal blood and bodily fluid, and ingestion of un/under-cooked meat. Various wildlife pathogens, including new retroviruses, have been detected in people who hunt and butcher wild animals in Central Africa (Wolfe et al 2004, 2005; Zheng et al 2010). Understanding the diversity of pathogens in wildlife in RoC aids in preventing or limiting the emergence of new infectious diseases and, thus, reduces the threat of pandemics.

PREDICT collaborated with the Government of RoC to build wildlife surveillance capacity. This partnership allowed PREDICT to evaluate the diversity of wildlife viruses with the potential for human infection, as well as to explore the implications of human and wildlife interactions on disease transmission and emergence. A better awareness of potential zoonotic viruses allows public health authorities and communities to consider measures to reduce risk of infection and to help with the rapid identification of viruses that may cross over from wildlife to humans.

FOR A SUMMARY OF THIS SECTION GO TO WWW.ROC.PREDICT.GLOBAL

PARTNERS

PREDICT partners in RoC included:

- African Parks
- Global Viral (GV)
- Global Viral Cameroon (GV-C)
- IUCN Elephant Bushmeat Pilot Project
- Laboratoire Nationale de Sante Publique/National Public Health Laboratory (LNSP/NPHL)
- Laboratoire de Diagnostique Vétérinaire de Brazzaville/Brazzaville Veterinary Diagnostic Laboratory (LDVB)
- Metabiota
- Ministère de l'Agriculture/Ministry of Agriculture
- Ministère de l'Economie Forestière et du Développement Durable/Ministry of Forestry Economics and Sustainable Development
- Ministère de la Défense Nationale/Ministry of National Defence
- Ministère de la Santé et de la Population/Ministry of Health and Population
- Ministre de la Recherche Scientifique et de l'Innovation Technologique/Ministry of Scientific Research and Technological Innovation
- National Institute of Allergic and Infectious Diseases, National Institute of Health, Rocky Mountain Labs (NIAID/NIH-RML)
- National Institute of Allergic and Infectious Diseases, National Institutes of Health, Vaccine Research Center (NIAID-VRC)
- Projet Nouabale-Ndoki (PNN)
- Université Marien NGOUABI/Marien NGOUABI University (UMN)
- Wildlife Conservation Society (WCS)
- World Health Organization Regional Office for Africa (WHO AFRO)
- USAID

MAJOR ACHIEVEMENTS

- Established a community wildlife surveillance system in areas of high risk for Ebola virus emergence by educating hunters on the best procedures for reporting animal carcasses found in the forest (see Success Stories for more information).
- Published evidence-based animal surveillance recommendations for a series of human Ebola virus outbreak investigations in Africa. Guidance was provided on susceptible target species and prioritization of assays to rapidly detect the presence of Ebola virus in animal reservoirs (see Success Stories for more information).

- Tested fruit bats from a bushmeat market for paramyxoviruses to investigate risk to humans associated with hunting and preparation and consumption of bats. Four samples yielded Henipavirus-related sequences that formed at least 3 distinct groups in the *Paramyxoviridae* family.
- Conducted research to optimize noninvasive sampling strategies for disease surveillance in gorilla populations in Central Africa (see Success Stories for more information).
- Enhanced disease surveillance capacity and coordination among animal and public health officials to ensure communication across health sectors. PREDICT coordinated the translation of classroom-based training into practical experience through facilitation of field surveillance activities led by teams from the Veterinary Diagnostic Laboratory Brazzaville (LDVB) and the National Public Health Laboratory (LNSP) and mentored by technicians experienced in wildlife surveillance and PREDICT protocols.

SUCCESS STORIES

Dead or Alive: Animal Sampling During Ebola Hemorrhagic Fever Outbreaks in Humans

PREDICT provided evidence-based animal surveillance recommendations for a series of human Ebola hemorrhagic fever outbreak investigations in Africa (Olson et al. 2012a), including information on susceptible target species, guidance on animal sampling for disease outbreaks occurring in resource-limited regions, and diagnostic assays to be prioritized to rapidly assess the presence of Ebola virus in animal reservoirs. Specific recommendations for targeted surveillance aimed to identify potential sources of transmission from animals to humans included: 1) investigation of all wildlife morbidity and mortality events; 2) sampling of animal carcasses (vs. live animals) for a higher likelihood of Ebola virus recovery; 3) surveillance of certain bat species using large sample sizes ($n > 100$); and 4) prioritization of dogs and pigs among domestic animals for screening for virus and antibodies (i.e. previous exposure; Olson et al. 2012a).

Building Hunter Networks to Help Detect Ebola Outbreaks in the Republic of Congo

The northern boundary of Odzala Kokoua National Park in the Republic of Congo is at high risk for Ebola emergence. PREDICT established a community wildlife surveillance network in the area by educating hunters on the best procedures for reporting animal carcasses found in the forest. The goal of this community-based surveillance system is to allow for continued, sustainable monitoring of potential outbreaks.

Hunters represent about 22% of the population in this highly susceptible region. The team visited 512 hunters in 27 villages that had previously experienced Ebola epidemics, reinforcing the importance of reporting wildlife morbidity or mortality events. During the project, hunter networks reported numerous gorilla carcasses prompting PREDICT teams to be mobilized for sampling.

Optimization of Noninvasive Methods for Wildlife Disease Surveillance in Nonhuman Primates

PREDICT scientists also conducted research to optimize noninvasive sampling techniques for conducting disease surveillance for wildlife taxa that are difficult to locate and capture (i.e. nonhuman primates). Simulation modeling was used to assess optimal sampling strategies for detecting fecal samples from gorillas in Central Africa. The authors simulated a number

of different sampling survey designs to identify which design maximized the number of fecal samples detected while also producing accurate estimates of gorilla population densities. The designs were evaluated for accuracy and cost and time efficiencies over a variety of different gorilla population densities and distributions (Olson et al. 2012b). A mixed sampling design, combining traditional transect and directed reconnaissance designs, maximized the detection of fecal samples and estimates of gorilla density, while targeted reconnaissance sampling maximized sampling efficiency but produced biased population density estimates (Olson et al. 2012b).

CAPACITY BUILDING

Training

PREDICT presented unique training opportunities for staff, collaborating ministries, the national public health laboratory, and the national veterinary diagnostic laboratory (Laboratoire de Diagnostique Vétérinaire de Brazzaville - LDVB). Training was provided to eight technicians from the National Veterinary Diagnostic Laboratory and staff from regional livestock and wildlife authorities on the use of PPE, wildlife capture and handling, and sample collection and storage.

This training was held prior to field surveillance activities led by the LDVB and the National Public Health Laboratory and was supported by PREDICT Cameroon staff. PREDICT mentored LDVB staff during the planning and execution of wildlife sampling. Brazzaville Veterinary Diagnostic Laboratory staff led the planning and execution of field surveillance activities, and local and central ministerial staff observed elements of the surveillance in action. Training conducted in the classroom was augmented through field-based sessions, which reinforced the didactic material and provided practical experience.

PREDICT also provided laboratory training to technicians based at the National Public Health Lab and from the Université Marien Ngouabi in Brazzaville. The trainings covered topics including surveillance techniques, wildlife capture and sampling, sample handling and transport, laboratory analyses and management, laboratory best practices, and database management.



Photo left: PREDICT team undertaking a training of staff from Brazzaville Veterinary Diagnostic Laboratory and National Public Health Laboratory on PREDICT sample collection protocols in Ouessou, RoC.

Photo right: Personal protective equipment demonstration by PREDICT in Ouessou, RoC for staff from Brazzaville Veterinary Diagnostic Laboratory and National Public Health Laboratory.



Common rufous-nosed rat (*Oenomys hypoxanthus*) sampled as part of PREDICT surveillance in Gombé Village.

Training was conducted at the National Public Health Laboratory in Brazzaville by project staff and collaborators from Tulane University, National Microbiology Laboratory, PHAC SPP, and Laboratory of Virology, NIAID/NIH RML. Additional training was conducted at the PREDICT laboratory in Yaoundé Cameroon. PREDICT demonstrated in-country capacity for PCR and its field portability to representatives of RoC Ministries of Health, Ministry of Forest Economy, Ministry of the Environment, Ministry of Defense, and Ministry of Agriculture.

Staff from PREDICT RoC also participated in training at the Pan-African Sanctuary Alliance (PASA) annual meeting in South Africa covering topics such as necropsy, new laboratory analytical techniques, introduction to disease risk analysis for primate reintroduction programs, disease contingency planning, and scientific writing and presentation skills.

Laboratory Infrastructure and Diagnostics

At the National Public Health Laboratory, PREDICT made investments in laboratory infrastructure with support from the NIH. PREDICT developed capacity for laboratory diagnostics, including detection of various strains of Ebola and Marburg viruses (i.e. Zaïre ebolavirus, Sudan ebolavirus, Côte d'Ivoire ebolavirus, Bundibugyo ebolavirus, and Marburg virus). This diagnostic panel was expanded to include yellow fever virus, poliovirus, dengue virus, Rift Valley fever virus, malaria, *Mycobacterium ulcerans*, measles virus, and monkeypox virus. Specimens were also tested at various partner laboratories including CIRME, Columbia University Center for Infection and Immunity (CII), University of California, Davis, and the INRB PREDICT laboratory in Kinshasa.

SURVEILLANCE

A range of high-risk human-wildlife interfaces were investigated in RoC (Figures 1 and 2). Sites and species targeted for wildlife surveillance were chosen based on risk of disease outbreaks from known pathogens, such as Ebola virus, and novel pathogens that could be transmitted from wildlife to people through hunting and butchering and contact in or around human dwellings in rural areas (Table 1). An additional area of focus included extractive industry sites. PREDICT coordinated with rapid bio-assessment missions and other US government agencies (NIAID/NIH Rocky Mountain Labs and PHAC Special Pathogens Program) conducting research in areas of priority for surveillance, in particular sites in areas impacted by extractive industries.

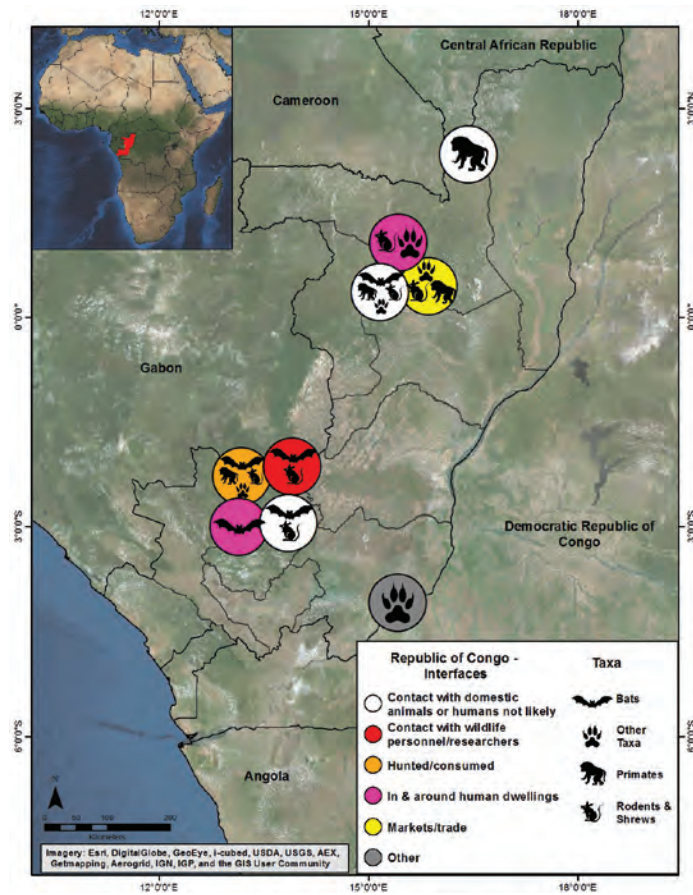


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

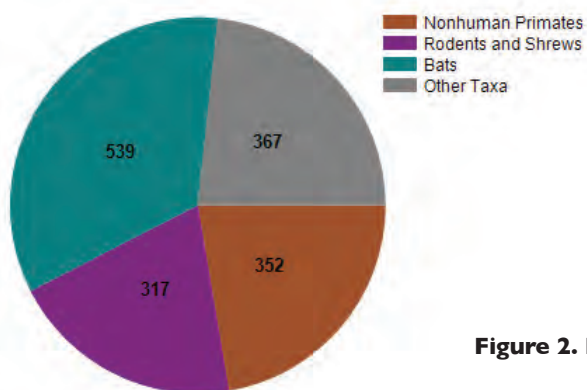


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Ecotourism and recreational activities	1	0	0	0
Extractive industries	0	59	73	0
In or near human dwellings	3	69	93	1
Hunted wildlife	91	34	54	248
Markets	88	20	43	115
Wildlife being studied	0	31	11	0
Zoos and sanctuaries	1	3	0	0
Pristine habitat	168	101	265	2
Other high-risk interfaces	0	0	0	1
Total:	352	317	539	367

PREDICT-RoC also investigated the best noninvasive sampling methods for free ranging primates, including optimal strategies for fecal sample collection from gorillas in the dense forests of Central Africa (see Success Stories for more information; Olson et al. 2013).

Community Hunter Wildlife Surveillance Network

A group of 285 hunters among ten villages aided the team in identifying bat roosting sites, including a number of roosts in caves and mines. Samples were also collected in villages and in markets from primate, swine, carnivore, and rodent carcasses destined for human consumption. This opportunistic collection included some species outside the priority wildlife taxa for PREDICT, such as duikers and hogs, because these species have been implicated in Ebola virus ecology.

PREDICT also established a community-based animal morbidity and mortality surveillance system in areas of high risk of Ebola emergence. A total of 512 hunters in 27 villages were visited in regions previously experiencing Ebola epidemics and were provided training on how to report wildlife morbidity or mortality events. Hunter networks reported numerous gorilla carcasses during the course of PREDICT, and teams were mobilized for sampling.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT-RoC reported key information on a number of suspected hemorrhagic disease outbreaks in humans and wildlife to the RoC Ministry of Health. During a suspected hemorrhagic fever disease event in Mokouangonda, PREDICT and WHO AFRO supported the MoH in their response by mobilizing a response team. This team conducted interviews and collected patient history, clinical data, epidemiologic information, and biological samples. Additionally, PREDICT mobilized four field teams in the areas surrounding the outbreak to collect samples from wildlife and conduct reconnaissance surveys to evaluate disease outbreak effects on large primates and other wildlife. PREDICT facilitated communication with other field partners, such as WCS and African Parks throughout the course of the project.

PREDICT also assisted with the response to an outbreak in Likuala, Northern Congo by facilitating the transport and testing of clinical samples at the Centre International de Recherche Médicales de Franceville (CIRMF). The samples were screened for Chikungunya, dengue, yellow fever, Rift Valley fever, West Nile, Zika, and O'nyong nyong viruses.

In addition, PREDICT participated in a workshop with government officials (MoH, Epidemiology and Hygiene Service, and Avian Influenza project), WHO, and local communities with the aim of improving reporting speed, transparency, and response during disease outbreaks.



Staff from Brazzaville Veterinary Diagnostic Laboratory and National Public Health Laboratory managing samples collected during PREDICT surveillance from bats and rodents in Gombé, RoC.

VIRUS DETECTION AND CHARACTERIZATION

Henipavirus-related Sequences in Fruit Bats from a Bushmeat Market in the Republic of Congo

Bats are hosts for the zoonotic paramyxoviruses Hendra virus and Nipah virus, which have caused severe disease outbreaks among humans and livestock. PREDICT investigated the risk of cross-species transmission of paramyxovirus associated with hunting, preparation, and consumption of bats in the Republic of Congo. Samples obtained from live straw-colored fruit bats (*Eidolon helvum*) captured by hunters for bushmeat were screened for paramyxoviruses (i.e. respirovirus, morbillivirus, and henipavirus) by PCR.

Viral sequences from 11 bats formed at least three distinct groups in the *Paramyxoviridae* family (Weiss et al. 2012). Henipaviruses cluster in-between these bat paramyxovirus sequences. Phylogenetic analysis revealed no spatial distinction between the sequences in the bats in the Republic of Congo and previous sequences detected in bats from Ghana, suggesting that various strains are exchanged over large distances by the migrating bats (Weiss et al. 2012). While there are no documented cases of human infection associated with bat paramyxoviruses in Africa, antibodies to henipavirus or henipa-like viruses have been detected in domestic pigs, which are amplifying hosts for people and may indicate exposure to virus in bat excreta (Hayman et al. 2011). Additional research on virus-host ecology and assessment of at-risk persons are needed to evaluate the zoonotic disease risk of these viruses.

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PHOTOS BY GORILLA DOCTORS



Rwanda has built a highly successful ecotourism industry, which brings both local community members and international tourists into daily contact with wildlife.

RWANDA

Rwanda has built a highly successful ecotourism industry that now represents one of the country's largest sources of foreign revenue. Ecotourism in Rwanda is largely centered on opportunities to view wild human-habituated mountain gorillas in Volcanoes National Park, which brings both local community members (park personnel) and international tourists into daily direct and indirect contact with mountain gorillas. Nyungwe National Park is an increasingly popular destination for tourists and is also home to human-habituated chimpanzees and 12 other primate species. Rwanda is also developing cave tourism, which brings tourists into close contact with roosting bats. Opportunities for wildlife contact in Rwanda are not restricted to national parks, however: in areas surrounding these national parks, as well as in urban, semi-urban, and rural communities, primates (e.g. vervet monkeys, blue monkeys, and olive baboons) range near and in people's homes and crops, and contact with bats and rodents in and around homes is also common, especially in more rural locations.

Rwanda is continental Africa's most densely populated country (World Bank 2014). Outside of urban centers, most Rwandans live in rural settings, in close contact with their domestic livestock. Use of land for subsistence crop and animal agriculture brings people into direct proximity to Rwanda's only remaining natural habitats (national parks). People enter parks illegally to hunt or collect firewood, and wildlife exit parks to forage on crops.

Rwanda is considered a geographical hot spot for zoonotic diseases, and these aforementioned circumstances present unique opportunities for the emergence of pathogens from wildlife that could pose a threat to human health. Therefore, there was an urgent need in Rwanda to strengthen collaborations among government ministries, universities, and private partners, including commercial enterprises involved with tourism and conservation, and to better integrate

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veterinary, agricultural, and public health services to enable disease surveillance at the interfaces among wildlife, domestic animals, and people. Through the PREDICT project, the US-based non-governmental organization Mountain Gorilla Veterinary Project (MGVP, Inc.) partnered with the University of California, Davis to survey wildlife for viral pathogens of zoonotic potential, build capacity through improvements in disease surveillance systems and diagnostic laboratory networks, operationalize One Health, and enhance zoonotic disease outbreak response preparedness. These efforts formed Rwanda's first-ever zoonotic disease surveillance system targeting wildlife at high-risk human-animal interfaces in areas of Rwanda that are most vulnerable to zoonotic disease emergence.

The Government of Rwanda is at the forefront of applying a One Health approach to issues of human and animal health importance. It established a One Health Steering Committee that involves the Rwanda Biomedical Center (the implementation arm of the Ministry of Health), the Rwanda Development Board (the implementation arm of the Office of the President), the Rwanda Agriculture Board (the implementation arm of the Ministry of Agriculture), and University of Rwanda's College of Veterinary Medicine and School of Public Health. Other relevant stakeholders, including the USAID Emerging Pandemic Threats PREDICT and RESPOND projects, USAID Mission Uganda, and the US Centers for Disease Control and Prevention (CDC), were also involved in the Steering Committee's formulation of a One Health policy statement that will be adapted by the relevant government ministries for implementation purposes. This endeavor, amongst other initiatives, such as OHCEA (One Health for Central and East Africa), which twins schools of veterinary medicine and public health in East and Central Africa with counterpart universities in USA, and the Rwanda One Health student club, will be important platforms for promoting and operationalizing One Health strategies that are essential in mitigating emerging pandemic threats in the country.

PARTNERS

In conjunction with the Government of Rwanda, PREDICT-Rwanda was implemented by the non-profit Mountain Gorilla Veterinary Project (MGVP, Inc.), which in partnership with the University of California, Davis, runs the Gorilla Doctors program in Rwanda, Uganda, and the Democratic Republic of the Congo.

Key local partners included:

- Rwanda Development Board/Tourism and Conservation (RDB): RDB is the government agency with jurisdictional authority for implementing all tourism and conservation activities, including wildlife management and research in Rwanda; PREDICT's chief point of contact was Dr. Antoine Mudakikwa, the veterinarian in charge of all wildlife health surveillance, research, and monitoring.
- Rwanda Agriculture Board (RAB): RAB is the government agency with jurisdictional authority for implementing all crop and animal agricultural, husbandry, and science

Straw-colored fruits bats (*Eidolon helvum*) were sampled all over Rwanda, including here, in the heart of Kigali.



PHOTOS BY GORILLA DOCTORS

initiatives aimed at improving food security in Rwanda. PREDICT's chief points of contact were Dr. Isidore Gafarasi, Head of RAB's Veterinary Extension Services, and Dr. Christine Kanyandekwe, RAB's Deputy Director General for Animal Resources.

- Rwanda Biomedical Center (RBC): RBC is the government agency with responsibilities for implementing all public health delivery and protection programs in Rwanda. Our chief point of contact was Dr. Thierry Nyatanyi, Head of the Epidemic and Infectious Diseases Unit.

Other partners included:

- USAID
- University of Rwanda's School of Animal Science and Veterinary Medicine: the University is committed to One Health capacity-building through didactic and experiential learning opportunities.
- The One Health Steering Committee of the Government of Rwanda: this committee was established and is led by the Ministry of Health, the Ministry of Agriculture, and RDB/ Tourism and Conservation Department.

MAJOR ACHIEVEMENTS

- Systematically collected samples from 1,447 live free-ranging, captive, and confiscated wildlife (503 nonhuman primates, 402 rodents, 504 bats, and 38 other wildlife species) at high-risk disease transmission interfaces in areas where wildlife have high levels of interaction with humans and domestic animals; this work was accomplished in close partnership with the Rwanda Development Board.
- Participated in the establishment of the Government of Rwanda's One Health Steering Committee to improve cross-sectoral systems and processes for human and animal disease outbreak response (see Success Stories for more information).
- Conducted extensive surveillance of potential zoonotic viruses in nonhuman primates in Rwanda (see Success Stories for more information).

PREDICT staff and a National University of Rwanda veterinary student at the Rwanda Agriculture Board Animal Health Extension station in Rubilizi, Kigali, obtaining samples from a bat that was found dead.



PHOTOS BY GORILLA DOCTORS

- Served as a member of the Government of Rwanda's Disease Outbreak Response Taskforce, helping inform the taskforce on the potential role of wildlife in the emergence and spread of zoonotic disease; PREDICT-Rwanda helped the taskforce consider potential wildlife contributions to a disease outbreak in cattle in Bugesera District, which later proved to be an outbreak of Rift Valley Fever.
- Established a large-capacity, secure biobank for long-term storage of wildlife samples.
- In partnership with the Rwanda Agriculture Board Animal Extension Services unit in Rubilizi, Kigali, PREDICT-Rwanda created

Rwanda's first BSL-2+ wildlife diagnostic laboratory. Laboratory personnel were trained on sample processing for application of molecular diagnostic techniques.

- Processed 632 nonhuman primate, bat, and rodent samples in the BSL-2 laboratory in Rwanda. PREDICT-Rwanda partnered with the Makerere University Walter Reed Project in Uganda to conduct broad-based testing of these samples for 18 viral families of pandemic potential, which led to the detection of both known and previously-unknown viral pathogens.
- Trained approximately 200 wildlife and government domestic animal veterinarians, veterinary and wildlife students, and other wildlife personnel in wildlife surveillance principles; biosafety; zoonoses; and primate, rodent, and bat sampling methods.

SUCCESS STORIES

Enhancing Understanding of Spillover of Pathogens between Wildlife and People

PREDICT-Rwanda conducted extensive surveillance for potential zoonotic pathogens in wildlife in Rwanda, particularly in primates; with primate viewing forming the backbone of Rwanda's highly successful ecotourism industry, the information now in hand regarding what pathogens primates carry that may pose a threat to people, and *vice versa*, will help inform decision-making by wildlife and tourism authorities. The genetic relatedness of great apes and humans has led to concerns about bi-directional spillover of pathogens between people and gorillas. Investigation into a respiratory outbreak in mountain gorillas in 2009 by PREDICT researchers revealed human metapneumovirus lineage B2 in affected individuals (Palacios et al. 2011). The source of the virus is unknown; however, the strain was most recently described in South Africa and likely was transmitted to the gorillas by humans, highlighting the potential for bi-directional spillover of pathogens (Palacios et al. 2011). Although human viewing of mountain gorillas for ecotourism is necessary for their conservation, minimizing the risk of zoonotic pathogen transmission between people and gorillas is critical for their long-term survival and a healthy tourism revenue stream for Rwanda.

Gorillas are poached for the wildlife trade, and confiscated orphaned gorillas are placed into captivity where they are provided constant contact and care by staff. In Rwanda, an orphaned Grauer's gorilla confiscated from poachers and held in captivity for more than two years developed oral lesions. Human herpes simplex virus Type 1 was detected in this gorilla (Gilardi et al. 2014). This study conducted by PREDICT and its partners was the first to document spillover of viruses from humans into Grauer's gorillas and provides further evidence of the bi-directional spillover of pathogens between people and gorillas (Gilardi et al. 2014).

Establishment of the Government of Rwanda's One Health Steering Committee

PREDICT-Rwanda participated in the establishment of the Government of Rwanda's One Health Steering Committee to improve cross-sectoral systems and processes for human and animal disease outbreak response. PREDICT helped provide a broad understanding of the role of wildlife in zoonotic disease outbreaks and explained available techniques for conducting wildlife pathogen surveillance at high-risk human-wildlife interfaces. The One Health Committee is comprised of representatives of the Rwanda Biomedical Center (RBC),

Gorilla Doctors' veterinarian, Jean-Felix Kinani, obtains samples for infectious disease screening from a mountain gorilla that was immobilized to remove a snare.



PHOTOS BY GORILLA DOCTORS

Rwanda Development Board (RDB), Rwanda Agriculture Board (RAB), EPT PREDICT and EPT RESPOND, University of Rwanda's Veterinary Medicine College and Medical College, Centers for Disease Control and Prevention (CDC), USAID Mission Rwanda, FAO, and WHO. PREDICT was invited to participate in the establishment and formulation of the 5-year strategic plan and a cross-sectoral One Health policy statement to be adapted by the relevant ministries in the social cluster.

Rolex Recognizes PREDICT Field Veterinarian's Vision for Wildlife Conservation

PREDICT Field Veterinarian Dr. Olivier Nsengimana received a 2014 Rolex Award for Enterprise in June 2014 in recognition of his achievement and future promise as a wildlife veterinarian in Rwanda. One of just five Young Laureates selected from among 1,800 nominees from around the world, Dr. Nsengimana will apply his award to a collaborative project with the Rwanda Development Board to repatriate illegally-kept Grey Crowned Cranes to rehabilitation centers and eventually back to the wild, where it is believed only 300-500 cranes remain. The knowledge and skills that Nsengimana gained in implementing PREDICT field surveillance activities, as well as the RDB's capacity for, and commitment to, implementing wildlife conservation and One Health initiatives like PREDICT, position Nsengimana and his Rwanda colleagues for success on this important project.

PREDICT staff and a National University of Rwanda veterinary student volunteer at a field sampling station in Nyungwe National Park.

CAPACITY BUILDING

Infrastructure Development

PREDICT-Rwanda established the first-ever wildlife virology laboratory at the Rwanda Agricultural Board in Kigali. The facility was equipped with BSL-2+ safety and diagnostic equipment for processing wildlife samples, including an isolation unit with a biosafety cabinet, equipment, and supplies for viral detection. Refurbishment of an existing generator and installation of an automatic generator switch ensured a steady power supply. This work was accomplished in close partnership with the Rwanda Agriculture Board Animal Extension Services unit in Rubilizi, Kigali. RNA extraction of more than 600 samples was conducted at the PREDICT-Rwanda lab and PCR was performed at the Makerere University Water Reed Project in Uganda through a newly established collaboration to improve capacity for a regional diagnostic laboratory network in East Africa.

Training

PREDICT-Rwanda conducted comprehensive training of wildlife and domestic animal veterinarians and veterinary and public health faculty and students on zoonotic disease outbreak investigation using PREDICT protocols and training modules covering personal safety during wildlife sampling, humane and effective capture and sampling of wildlife, and disease outbreak response and preparedness. As a result of this training, approximately 200 people gained the knowledge and skills to assist the Government of Rwanda with investigation of the role of wildlife in future human or animal disease outbreaks.



PHOTOS BY GORILLA DOCTORS



PREDICT conducted the first training for veterinarians in Rwanda on proper and safe sampling of wildlife, laboratory and biosafety protocols, and disease outbreak response. Participants included the Tourism and Conservation Department of the Rwanda Development Board, Rwanda Agriculture Board, University of Rwanda College of Veterinary Medicine, and Gorilla Doctors.

PREDICT-Rwanda also served as a regional training hub for nonhuman primate surveillance for the PREDICT project in east-central Africa, training the country coordinator and staff scientists from PREDICT-Tanzania on safe primate capture and sampling, thereby further strengthening regional and transboundary networks for disease surveillance.

Operationalizing One Health

PREDICT-Rwanda assisted with formation of the Government of Rwanda's One Health Steering Committee, including development of a five year strategic plan and a cross-sectoral ministerial One Health policy statement. PREDICT's active participation in this committee led to an improved understanding of the role of wildlife in zoonotic disease outbreaks and strategies for conducting surveillance targeted at high-risk human-wildlife disease transmission interfaces.

SURVEILLANCE

PREDICT systematically collected samples from 1,447 free-ranging, captive, and confiscated wildlife (504 bats, 402 rodents, 503 nonhuman primates, and 38 animals from other wildlife taxa) throughout Rwanda, targeting areas surrounding national parks, as well as urban centers and semi-urban communities (Figures 1 and 2). Surveillance was targeted at high-risk disease transmission interfaces, including ecotourism, agricultural settings, in and around human dwellings, sanctuaries, and hunted and consumed wildlife (Table 1).

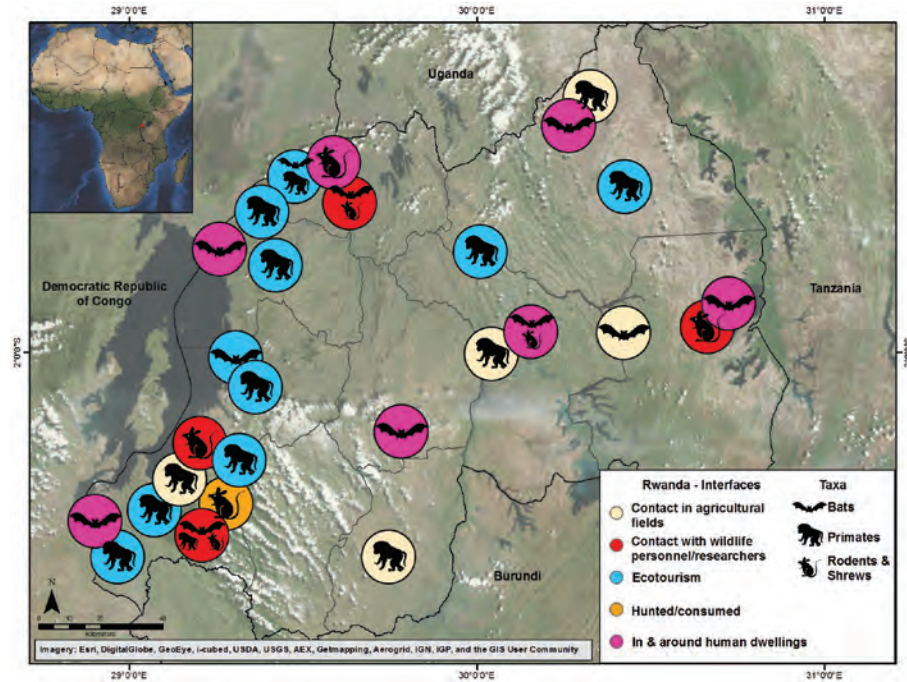


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

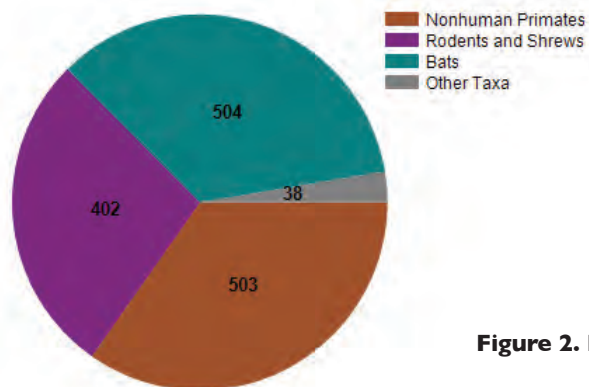


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	27	0	25	0
Ecotourism and recreational activities	174	0	169	1
Extractive industries	0	0	10	0
In or near human dwellings	39	64	235	0
Hunted wildlife	9	13	0	2
Wildlife trade	13	0	0	0
Wildlife being studied	1	184	0	0
Protected areas	219	14	36	0
Zoos and sanctuaries	10	0	0	0
Other high-risk interfaces	11	127	29	35
Total:	503	402	504	38

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT-Rwanda served as a member of the Government of Rwanda's Disease Outbreak Response Taskforce, helping guide the taskforce on the potential role of wildlife in the emergence and spread of zoonotic disease. For example, PREDICT helped the taskforce consider potential wildlife contributions to a disease outbreak in cattle in Bugesera District, which later proved to be an outbreak of Rift Valley fever.

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In Tanzania, rapid population growth and land conversion have led to greater sharing of resources and increased interactions among wildlife, domestic animals, and humans.

PHOTOS BY LIZ VANWORMER (LEFT) AND BY WOUTRINA SMITH (RIGHT)

TANZANIA

In Tanzania, human livelihoods are often directly dependent on natural resources. Rapid population growth has increased land-use change, the demand for natural resources, and the potential for contact between wildlife and people. Tanzania has diverse wildlife-livestock-human interfaces, which offer ideal targets for zoonotic disease surveillance. Tanzania is home to millions of wild animals, many of which migrate across borders to other countries and ecosystems. Ongoing human and environmental changes are altering landscapes and connections between people and animals. For example, in the more arid areas of the Rift Valley, which join the Congo Basin in the west, water diversion and land conversion for agriculture coupled with continued population growth are restricting once vast livestock grazing areas for traditional livestock keepers (Copolillo et al. 2008; Walsh 2012). The result is an increase of livestock populations on remaining grazing land, often bordering villages and wildlife protected areas. The increasing density of livestock near wildlife-rich protected areas coupled with water scarcity may change the dynamics of current zoonotic threats and may facilitate transmission of diseases of pandemic potential across this interface (Mazet et al. 2009). Additionally, Tanzania possesses many islands of rainforest habitat, most notably the Eastern Arc Mountains, which are biodiversity hotspots due to the high degree of endemism and small mammal diversity (Burgess et al. 2007). These remnant forests provide an exceptional opportunity to assess zoonotic pathogen potential in rodent, bat, and primate species. Rapidly growing urban centers like Dar es Salaam on the Indian Ocean coast present another unique environment where pathogens from bats and rodents have the potential to be transmitted to and spread in dense human populations.

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Despite the wealth of biodiversity and close interaction of human and animal populations at these interfaces, Tanzania's capacity to conduct wildlife disease surveillance at the regional and national level is still developing. Existing collaborations between Sokoine University of Agriculture (SUA) and the ministries and institutions responsible for livestock, wildlife, and human health enabled the PREDICT team to increase and expand wildlife surveillance activities at high-risk wildlife-livestock-human interfaces throughout the country. These collaborations were strengthened through PREDICT surveillance work, as well as capacity building activities, in partnership with the Tanzania Wildlife Research Institute and other local stakeholders.

The PREDICT Project in Tanzania, in collaboration with in-country partners, conducted active surveillance of viruses of wildlife at critical human-wildlife interfaces. Thorough site assessment and sample collection from diverse bat, rodent, and nonhuman primate species allowed the PREDICT team to examine the potential for zoonotic transmission at many high-risk disease transmission interfaces.

LOCAL PARTNERS

In Tanzania, PREDICT established partnerships with national ministries, universities, and NGOs to conduct surveillance activities and inform key stakeholders on progress on a quarterly basis. Partners in Tanzania and East Africa included:

- Sokoine University of Agriculture (SUA)
- USAID
- Ministry of Livestock and Fisheries Development
- Ministry of Health and Social Welfare
- Ministry of Natural Resources and Tourism
- Tanzania Wildlife Research Institute (TAWIRI)
- Tanzania National Parks (TANAPA)
- National Institute of Medical Research (NIMR)
- Muhimbili University of Health and Allied Sciences
- Central Veterinary Laboratory and Regional and District Veterinary Offices
- Centers for Disease Control and Prevention Laboratory, Dar es Salaam
- Centers for Disease Control Global Disease Detection Group, Nairobi, Kenya
- Makerere University Walter Reed Project (MUWRP), Uganda

Udzungwa red colobus was one of the diverse primate species sampled at ecotourism interfaces in the Udzungwa Mountains.



PHOTO BY PREDICT TANZANIA TEAM

MAJOR ACHIEVEMENTS

- Implemented the first systematic and national-level viral surveillance approach for wildlife in Tanzania (see Success Stories for more information).
- Trained a One Health Workforce by providing a variety of educational and training opportunities at multiple levels (see Success Stories for more information).
- Developed and refined the PREDICT local media surveillance system to identify reports of human and animal disease in local Tanzanian news sources not currently integrated into digital disease detection systems like HealthMap, providing proof of concept for the expansion of local media surveillance to seven other PREDICT countries.
- Partnered with CDC representatives and local district chief medical officers to plan paired human-animal surveillance activities at sugar cane plantations in central Tanzania. Pilot data from this collaboration will provide information on the risk of viral transmission from rodents to sugar cane plantation workers and could spur future One Health programs.
- Conducted 319 active wildlife sampling events, and collected samples from a total of 1,753 animals (650 bats, 632 rodents, 251 nonhuman primates, and 220 samples from other taxa, primarily samples collected from bushmeat).
- Developed the first viral detection laboratory for wildlife in Tanzania, trained laboratory technicians at Sokoine University of Agriculture (SUA) in consensus-based PCR diagnostics, and initiated screening for arenaviruses, a high priority viral family due to the detection of novel arenaviruses in commensal rodents in Tanzania.
- Increased government, non-governmental organization, and community stakeholder awareness of approaches to prevent disease transmission at human-wildlife interfaces through presentations and distribution of calendars with educational messages on zoonotic diseases.
- With support from the Emerging Pandemic Threats DELIVER project, installed a liquid nitrogen generating plant in south-central Tanzania to strengthen cold chain for biological materials and wildlife samples to enhance local disease surveillance activities.

SUCCESS STORIES

Building SMART Surveillance in Tanzania

PREDICT implemented the first systematic and national-level viral surveillance approach for wildlife in Tanzania, improving awareness of high-risk human-animal interfaces, targeting animal sampling in key taxa at prioritized sites representing Tanzania's biological and geographic diversity and building the infrastructure and human resource base for the detection of emerging pathogens of pandemic potential.

Further, PREDICT-Tanzania partnered with diverse stakeholders at multiple levels of Tanzanian health systems, including community game officers, health workers, livestock extension officers, university researchers, veterinary professionals, and ministry level officials to characterize disease transmission risk, conduct wildlife surveillance activities, and share results. In Tanzania, PREDICT incorporated indigenous knowledge and participation, local media systems, and online intelligence to design and optimize surveillance activities reflecting the country's broad ecological and behavioral diversity.



Photos: A maize field crop raiding interface where diverse species of rodents were sampled (left). A PREDICT field team member collects an oral swab sample from an anesthetized rodent captured at an agricultural market.

In 2009, preliminary capacity assessments identified few national and donor investments in wildlife disease surveillance, and those that did exist primarily emphasized known diseases of conservation concern. Outside of major urban centers, limited infrastructure existed to support collection of wildlife samples for molecular diagnostics. By January 2014, liquid nitrogen cold chain capability was extended to central areas of the country, and personnel from universities, the Tanzania Wildlife Research Institute (TAWIRI), government health workers, veterinary and livestock extension officers, and community members were trained and actively participated in the identification of priority human-animal interfaces and in safe animal capture and sampling of high-risk wildlife groups.

Strategic. Working at the community level with village councils, elders, government representatives, and wildlife professionals and at the global level through real-time disease outbreak alerts, PREDICT-Tanzania characterized human-animal interfaces by type of risk and strategically prioritized interfaces for surveillance to ensure coverage of high-risk taxonomic groups and a diversity of human-animal contact.

Measurable. PREDICT-Tanzania successfully sampled over 1700 animals from 12 areas in Tanzania representing diverse interfaces for human-animal contact and engaged with stakeholders in surveillance and risk reduction activities. PREDICT-Tanzania also tested priority specimens from over 1,200 individuals, building the first baseline data on potential emerging viral threats from wildlife to vulnerable populations.

Adaptive. PREDICT-Tanzania used site assessments and feedback from diverse stakeholders to support and adapt surveillance to interfaces and locations of highest risk for disease emergence and spillover. Surveillance of local media sources also identified disease reports, risky behaviors, and human-animal contact used to adapt surveillance strategies.

Responsive. PREDICT-Tanzania cooperated closely with local and regional partners to coordinate surveillance with existing wildlife, livestock, and human disease surveillance and monitoring activities. In collaboration with veterinary researchers from the Tanzania Wildlife Research Institute, PREDICT sampled bats in northern Tanzania's fragmented forests. At the request of the Centers for Disease Control and Prevention's Global Disease Detection group, PREDICT sampled rodents in the Kilombero Valley as a first step towards paired human-animal disease surveillance and assessments of viral sharing and occupational hazard in sugarcane plantations.



PREDICT-Tanzania and Tanzania Wildlife Research Institute team members conduct fruit bat trapping and sampling at Muheza in Tanga region.

Targeted. PREDICT-Tanzania targeted surveillance at interfaces where people have high contact with key wildlife groups. In close coordination with the surveillance and pathogen detection teams, priority samples from each animal were identified according to interface and potential transmission pathway and tested for viral families representing potential spillover and pandemic threat.

Training a One Health Workforce

In Tanzania, PREDICT provided a variety of educational and training opportunities at multiple levels, from community members to university students and health professionals. A total of 110 individuals (74 male, 36 female), including project research staff, were trained in topics ranging from disease surveillance (animal capture and handling, sample collection, and cold chain) to laboratory systems (biosafety, diagnostics, and information management), and disease prevention, enhancing Tanzania's disease surveillance system and One Health workforce.

PREDICT utilized a cooperative and participatory framework for surveillance site selection, working with local community members and district game and livestock officers to identify locations of wildlife habitat and collect information on human-animal contact to

characterize interfaces. In return, PREDICT provided training to an estimated 19 district level officials and community members in zoonotic disease transmission and prevention and on-the-job training in safe animal handling and sampling to multiple high-risk occupational groups (game officers, wildlife professionals, veterinarians, livestock officers, and hunters).

In partnership with RESPOND's One Health Central East Africa (OHCEA) University Network, PREDICT trained 47 future One Health leaders and scientists from Sokoine University of Agriculture and Muhimbili University of Health and Allied Sciences. Participants received hands-on field training in biosafety, safe wildlife capture and sampling, cold chain management, and One Health surveillance approaches. Training was also conducted in Morogoro, Tanzania at the SUA campus, including an overview of diagnostic methods for detecting zoonotic and emerging disease threats.

PREDICT sponsored staff participation in multiple capacity building opportunities, including:

- Wildlife Capture Africa course in Zimbabwe focusing on the safe use of chemical and physical restraint in wildlife immobilizations.
- Professional exchange and training for Tanzania laboratory technicians from SUA at Makerere University Walter Reed Project (MUWRP) in Uganda on DNA/RNA extraction, PCR, and data management to standardize pathogen detection regionally.
- Molecular diagnostic training for a SUA laboratory technician at the UC Davis One Health Diagnostic and Surveillance Laboratory in California on viral nucleic acid extraction, PCR techniques, consensus PCR viral family testing, and information management.
- One Health Summer School training at Sokoine University of Agriculture organized by Southern African Centre for Infectious Disease Surveillance (SACIDS) focused on the One

Health approach, disease and vector ecology, molecular epidemiology, biosafety, disease surveillance, and outbreak investigations.

- Safe capture, handling, and sample collection training for nonhuman primate disease surveillance with the PREDICT-Rwanda and Mountain Gorilla Veterinary Project field staff.
- Good Clinical Practices courses for researchers at the National Institute for Medical Research (NIMR), organized by Family Health International (FHI) and Kenya AIDS Vaccine Initiative Project (KAVI) Institute.

As a result of these training investments, PREDICT contributed to an enhanced social network of human resources for disease surveillance in Tanzania and encouraged greater transdisciplinary cooperation in the health system among Tanzania's future leaders and scientists.

CAPACITY BUILDING

Infrastructure improvements

At the Sokoine University of Agriculture's Faculty of Veterinary Medicine in Morogoro, Tanzania, PREDICT developed the first viral detection laboratory for wildlife in the country. The laboratory is equipped with trained personnel and advanced molecular technology to screen wildlife samples for known and unknown viruses within priority viral families (e.g. arenaviruses) and genera of potential pandemic potential and is networked to regional and global laboratory diagnostic centers of excellence in Uganda and the US for support and confirmatory testing. Technicians at the SUA lab regularly consult and engage in refresher trainings with mentors in the US with telemedicine-based knowledge and skill transfer, enabling acquisition and adoption of new techniques, protocols, and diagnostic testing on demand.

Additionally, with support from the EPT program partner DELIVER, PREDICT-Tanzania strengthened capacity for disease surveillance, sample collection, and storage through installation of a liquid nitrogen generating plant, the sole source of liquid nitrogen in the South-central area. The liquid nitrogen generator provides the means for maintaining cold-chain during sampling efforts and has enhanced capacity for other surveillance activities in Tanzania.

Operationalizing One Health

One Health research and disease surveillance capacity was improved nationally in cooperation with 10 partner organizations and agencies, including the Tanzania Wildlife Research Institute (TAWIRI), and through training programs to improve wildlife surveillance and disease diagnostics. Sharing of PREDICT activities and results with National Institute for Medical Research, Ministry of Livestock and Fisheries Development, and Tanzania Wildlife Research

PHOTO BY PREDICT TANZANIA TEAM



One Health Central and Eastern Africa University Network Students at a rodent capture and sampling training with PREDICT Tanzania team members.



PREDICT team members assess a fruit bat colony near human dwellings in Kilombero Valley in central Tanzania.

Institute increased discussion and awareness of wildlife viral surveillance and detection, promoting opportunities for future one health surveillance initiatives.

PREDICT-Tanzania also worked with Sokoine University of Agriculture and the Health for Animals and Livelihood Improvement (HALI) project to expand the One Health Workforce in Tanzania through a center of excellence in ecosystem and wildlife health. PREDICT and RESPOND in Tanzania effectively catalyzed wildlife health momentum at Sokoine University, where plans are in place to offer a new master's program in wildlife and ecosystem health, building on current graduate programs in One Health and molecular biology supported through the Southern African Centre for Infectious Disease Surveillance (SACIDS). Through these programs, veterinary medicine, agriculture, animal science, forestry, wildlife, and social science expertise will support transdisciplinary education and training using a One Health approach, with options for field-based training at the HALI project Iringa station, PREDICT Tanzania's surveillance team base. This center will provide a long-term training ground for students, faculty, researchers, and government personnel in One Health approaches to solving complex problems and will foster and encourage greater local, regional, and national cooperation and coordination of surveillance and health research.

SURVEILLANCE

PREDICT conducted wildlife surveillance at a number of high-risk wildlife-human contact interfaces in the following areas in Tanzania (Figure 1):

- South-central (Eastern Arc Mountains – Udzungwa Mountains National Park and surrounding forest reserves and villages, Kilombero Valley, Ruaha National Park and surrounding areas, Mbeya region, and Mbinga district)
- North-west (Geita – Industrial and artisanal mining areas)
- North-east (Eastern Arc – Amboni caves, Tanga region, and surrounding areas)
- Eastern (Coastal, Dar es Salaam and surrounding areas, Morogoro, and Mtwara)

High-risk interfaces targeted for wildlife pathogen surveillance in Tanzania included (Figure 2; Table 1):

1. Hunting/bushmeat interfaces where wild animals, most commonly rodents, ungulates, and carnivores, are consumed after being hunted, killed, and slaughtered under poor sanitary conditions;
2. Crop raiding interfaces where wild animals interact with humans by feeding on crops in local agricultural fields or in fruit and vegetable markets;
3. Peri-domestic settings and areas near extractive industries where wild animals (i.e. rodents and bats) commonly interact with humans in and around dwellings, villages, urban centers, and work sites or camps;
4. Ecotourism interfaces where tourists from around the world travel to see wildlife in unique environments including savannas, forests, mountains, and caves; and

- High traffic interfaces where construction of infrastructure including roads, railways, and buildings has enhanced human encroachment into wildlife habitat, and the rapid flow of people could facilitate rapid spread of zoonotic pathogens.

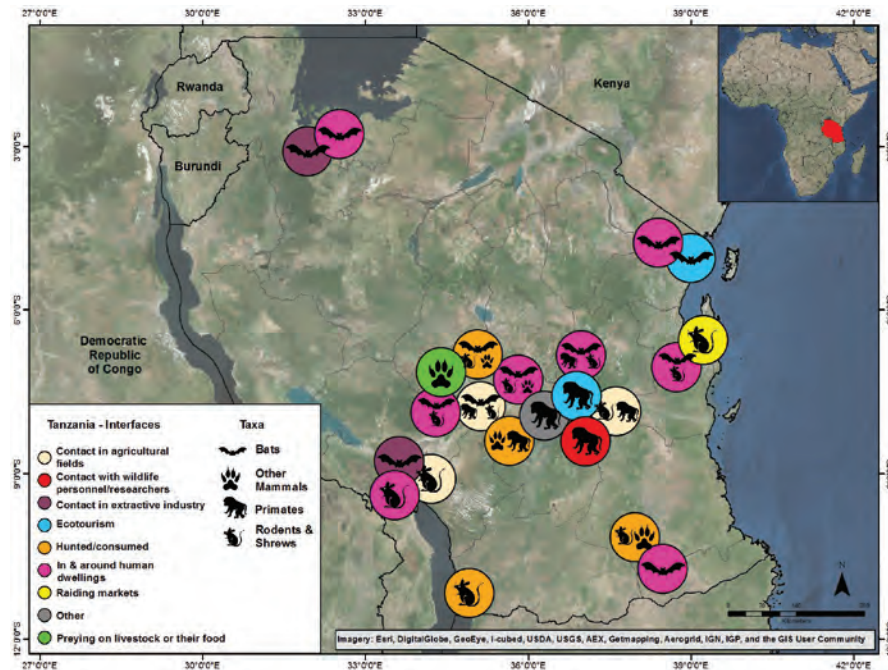


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

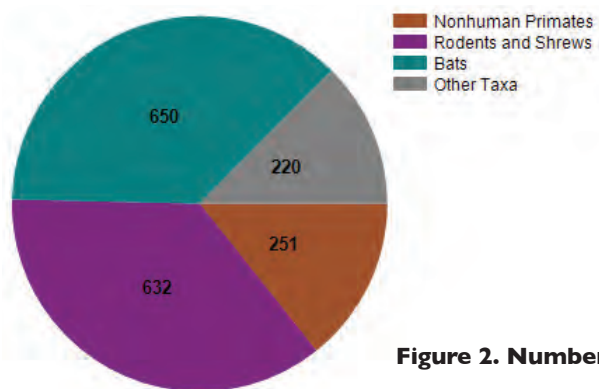


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	17	247	0	1
Ecotourism and recreational activities	30	0	59	0
Extractive industries	0	0	48	2
In or near human dwellings	22	306	543	7
Hunted wildlife	4	70	0	195
Wildlife preying on livestock or their food	0	0	0	15
Wildlife raiding markets	0	9	0	0
Protected areas	122	0	0	0
Other high-risk interfaces	56	0	0	0
Total:	251	632	650	220

Bushmeat hunting and consumption project. The PREDICT Tanzania team launched a bushmeat surveillance study in the Ruaha Ecosystem of South-central Tanzania within nine villages where both legal and illegal hunting are practiced. Working with a community surveillance network, PREDICT collected muscle and organ tissue samples from over 200 fresh-killed wild animals. The study revealed that consumption of bushmeat in the area is very common, with animals hunted and slaughtered under poor sanitary conditions, which may expose hunters to wildlife pathogens. Specimens from hunted animals will be tested for priority viral families using the consensus PCR approach. Additionally, we are conducting a survey of hunters and bushmeat consumers in the villages to assess the behavioral and socioeconomic factors associated with subsistence poaching in the area. Data obtained from this study will improve the understanding of the subsistence poaching in the area along with factors potentially implicated in zoonotic disease transmission risk.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

The PREDICT Tanzania team communicated with the Tanzania Wildlife Research Institute (TAWIRI), Ministry of Health and Social Welfare, and Ministry of Livestock and Fisheries Development to offer support in investigating numerous outbreaks in wildlife, domestic animal, and human populations, including outbreaks of African Swine Fever in Mbeya, undiagnosed illnesses in people in Gombe, and an outbreak of unknown origin killing goats and chickens on Musira Island in Bukoba. PREDICT surveillance and diagnostic support was not utilized in outbreak investigation and response efforts, but communications were strengthened with government partners on integrating PREDICT wildlife disease investigation support in national disease outbreak response planning.

The PREDICT Tanzania team collaborated with Tanzania National Parks (TANAPA) to jointly sample giraffe in Ruaha National Park, which were showing signs of an undiagnosed skin disease. PREDICT and TANAPA worked together to use advanced molecular diagnostics (deep sequencing) to identify the cause of this disease.

PHOTO BY PREDICT TANZANIA TEAM



PREDICT Tanzania director Professor Rudovick Kazwala (bottom right) works with a team of researchers from Tanzania National Parks and Sokoine University of Agriculture to safely collect samples from an anesthetized giraffe.

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In Thailand, rapid ecological changes threaten its biodiversity including several changes that are known to influence disease emergence, such as deforestation, agricultural intensification, mining, and wildlife hunting and trade.

PHOTOS BY WCS VIETNAM (LEFT), PATTAPOL MANEEON (RIGHT)

THAILAND

Thailand is the 20th most populous country in the world with approximately 68 million people (Geoba.se 2014). It is located within two major biogeographical regions, the Indochinese region in the North and the Sundaic region in the South – making it a hotspot for biodiversity. Thailand is facing rapid ecological changes that threaten its biodiversity, including several changes that are known drivers of disease emergence such as deforestation, agricultural intensification, mining, wildlife hunting, and wildlife trade.

Forested area in Thailand declined significantly from 61% to 34% of the country's landmass between 1945 and 1975. When last measured in 2011, it made up only 37% of the land area (Word Bank 2014). Historically, deforestation in Thailand was the result of rapid population growth and also an agricultural policy, which encouraged road construction and prevented many Thai citizens from securing property, thus forcing them to farm in the forests (Patarasuk et al. 2012). In the 1980s, the government took steps to reduce the rate of deforestation, including tree-planting initiatives, leases of degraded forest land for logging plantations, and a complete ban on commercial logging (Delang 2002).

Already, mammal diversity has decreased rapidly in the small, isolated forest fragments remaining after land conversion (Gibson et al. 2013). There are 335 species of mammals documented in Thailand (Duengkae 2011; Soisook 2011), representing nearly 3/5 of the known mammal species in Southeast Asia (Francis 2008). Many of the mammal species found in Thailand are bats, which account for 38% (138 species) of the total number of mammals. Eleven families and 45 genera of bats have been recorded from which 21 species are frugivores, 116 species are insectivores, and one species is carnivorous (Soisook 2011). Rodents are the second most common group of mammals, accounting for 25% of Thailand's mammal species (ONEP 2008).

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Thailand is a central hub for the illegal wildlife trade in Southeast Asia and, due to its geographic and economic position, is a crucial site for trade routes and therefore potential disease spread. Due to the scale of domestic animal production in Thailand, there is a high likelihood of zoonotic disease transmission among wildlife, domestic animals, and humans. Livestock account for about 2.5 percent of the GDP of Thailand (FAO 2005). Approximately 10 million pigs, 27 million ducks, and 281 million chickens, destined for local consumption and export, circulate within Thai farms.

Highly Pathogenic Avian Influenza H5N1 was first confirmed in Thailand in 2004. Since then, the virus has persisted as a major threat to human health and the poultry industry. It was generally hypothesized that persistence of the virus was due to traditional backyard chicken and free-range duck raising systems, which allow birds to roam freely around households, increasing their proximity to people and other animals. In late 2004, the Thai Government initiated a comprehensive surveillance program for influenza viruses, including H5N1 in traditional poultry rearing systems and also in wild bird populations by the Department of National Parks, Wildlife and Plant Conservation (DNP). This program represents the first systematic surveillance for wildlife pathogens conducted by the Thai Government.

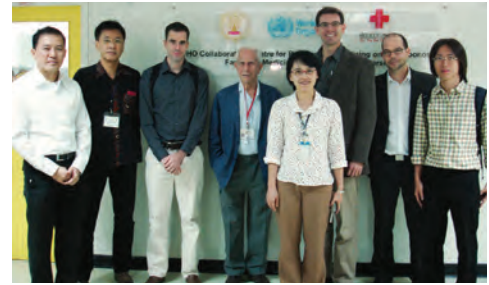
Prior to PREDICT, national wildlife disease surveillance programs in Thailand focused mainly on diseases associated with previous outbreaks in people, including avian influenza. Surveillance for other emerging zoonoses has largely been conducted through research performed by universities and other sectors. For example, researchers at Chulalongkorn University found evidence of exposure to Lyssaviruses (Lumlertdacha et al. 2005) and detected Nipah virus (Wacharapluesadee et al. 2005) in bats in Thailand. Surveillance for Nipah virus in bats has continued since this study, despite no reports of outbreaks in humans or pigs in Thailand. Thailand's wildlife diversity, especially its diversity of bats, in combination with high rates of deforestation and other anthropogenic activities (e.g. agriculture and other extractive industries and wildlife trade) make it a high-risk country for emerging pathogens of wildlife-origin in humans and other animals. In Thailand, PREDICT enhanced capacity for early detection of disease threats in wildlife and assisted with operationalizing One Health at a government and on-the-ground implementation level.

PARTNERS

The PREDICT project was implemented in Thailand as the collaborative work of EcoHealth Alliance, USAID, and Thai partners, including Faculty of Medicine, Chulalongkorn University; Faculty of Forestry, Kasetsart University; and the Department of National Parks, Wildlife and Plant Conservation (DNP).

Local Partners included:

- WHO Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University
- Faculty of Forestry, Kasetsart University
- Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment
- Bureau of Disease Control and Veterinary Services, Department of Livestock and Development (DLD), Ministry of Agriculture and Cooperatives



PREDICT Thailand collaborators from Chulalongkorn, Kasetsart University, EcoHealth Alliance, and Department of National Parks.

- Bureau of Emerging Infectious Diseases, Department of Disease Control, Ministry of Public Health
- Armed Forces Research Institute of Medical Sciences in Thailand and the U.S.
- Zoo Park Organization of Thailand
- National Institute of Animal Health, DLD, Ministry of Agriculture and Cooperatives
- The Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals (MOZWE), Mahidol University

MAJOR ACHIEVEMENTS

- Supported the development of the government's DNP first coordinated wildlife disease surveillance project to characterize the viral pathogen diversity in macaque populations (see Success Stories for more information).
- Discovered a novel group C Betacoronavirus in dry bat guano harvested as fertilizer. PREDICT staff in Thailand worked with managers and monks overseeing the guano mining area to better understand and characterize the risk of viral exposure to guano miners. In addition, PREDICT collaborated with the Emerging Pandemic Threats (EPT) PREVENT project on the design of an in-depth questionnaire to characterize the risk of human exposure and to assess strategies to reduce risk (see Success Stories for more information).
- Established One Health network of researchers conducting wildlife disease surveillance from Faculty of Medicine, Chulalongkorn University; Faculty of Forestry, Kasetsart University; and Department of National Parks. As part of this collaboration, personnel with expertise in all three sectors (i.e. human, wildlife, and domestic animal) have participated in active surveillance for pathogens in bats and nonhuman primates.
- Improved in-country capacity for wildlife disease surveillance through implementation of standardized animal sampling protocols to ensure safe wildlife handling. Trained 171 personnel (37% women) in PREDICT protocols and PPE.
- Established program for human pathogen testing using PREDICT protocols at Chulalongkorn University Hospital. The PREDICT diagnostic laboratory protocols are being used at the request of clinicians for the differential diagnosis of symptoms (including fevers of unknown origin and undiagnosed encephalitides) when traditional diagnostic methods fail.

- Developed laboratory network for H7N9 influenza virus and Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) testing and outbreak preparedness. Following the outbreak of H7N9 in China in 2013, the Ministry of Science initiated development of a diagnostic laboratory network for disease outbreak response preparedness. With the support of the Ministry of Science, diagnostic assays and protocols to investigate suspect cases of H7N9 and MERS-CoV were prepared for all of the hospitals in Thailand.
- Transferred technology and optimized PREDICT diagnostic methods (PCR assays for 24 viral families and genera) at the WHO Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University laboratory.
- Implementation the first Next-Generation Sequencing for pathogen discovery from wildlife samples in Thailand at Chulalongkorn laboratory.
- Supported the Thailand Government initiative, led by Mahidol University and the Zoological Park Organization, Thailand (ZPO), for the surveillance of MERS-CoV in camels in Thailand. Provided a two-day training session on MERS-CoV transmission, PPE, and laboratory assay support.
- Discovered several novel viruses, including coronaviruses, paramyxoviruses, and astroviruses in bats, rodents, and nonhuman primates.

SUCCESS STORIES

Macaque disease surveillance with the Department of National Parks – from Training to Project Implementation

PREDICT supported the development of the government's (DNP) first coordinated wildlife disease surveillance project to characterize the viral pathogen diversity in macaque populations.

Training. This activity included a three-day, hands-on training workshop for 78 participants, including several DNP field rangers and veterinarians. The first day of the workshop focused on the general knowledge of emerging infectious diseases (EIDs); zoonoses from wildlife; biosafety on animal handling and sampling; and methods for bat and primate capture, handling, and restraint. The second day covered material on the biology of bats and nonhuman primates, the diversity of the species in Thailand, bat ecology, sampling techniques, and PPE demonstration and practice. The last day included a hands-on training workshop in the field for bat capture and sample collection and demonstration for the safe handling and sample collection from macaques. The workshop training was overseen by EcoHealth Alliance, the PREDICT Thailand Country



PREDICT workshop for wildlife rangers and field personnel at DNP office, Bangkok February 2013.



PHOTOS BY DEPARTMENT OF NATIONAL PARKS

Coordinator, and a staff member of the USAID/Regional Development Mission for Asia who also presented an overview on the EPT program.

Participants attending the field training, February 2013.



PHOTO BY DEPARTMENT OF NATIONAL PARKS

Surveillance Implementation. Following the training, DNP staff sampled 50 long-tailed macaques (*Macaca fascicularis*) at Chakan cave, a PREDICT surveillance site, where extensive

surveillance in bat populations had been conducted the year before. At this site, macaques share the same habitat as bats and will often enter caves that support large bat populations. Similarly, humans frequent these caves, and the Khao Chakan Temple is a major tourist destination where people often feed and closely interact with the macaques. The PREDICT Thailand laboratory with support from the Thai government conducted PCR testing for herpesviruses, arenaviruses, paramyxoviruses, flaviviruses, hantaviruses, filoviruses, coronaviruses, retroviruses, influenza viruses, henipaviruses, bunyaviruses, poxviruses, bocaviruses, seadonaviruses, and astroviruses. A number of viruses were detected, including herpesvirus B, *Macaca fascicularis lymphocryptovirus*, and astroviruses. Partial sequences were generated and characterized for these viruses.



PHOTO BY PATTAROL MANEEON

Training on macaque sample collection.

undergoing relocation by DNP because they had become habituated and a nuisance to campers in the national park. Samples were screened for pathogens to inform on the health status of the translocated individuals and the potential risk to campers in the national park.

Lasting Change. As a result of these efforts, pathogen surveillance in macaques is now conducted annually by DNP, and the DNP deputy director has requested this surveillance be expanded to more areas of the country. PREDICT-Thailand in collaboration with the DNP sampled an additional 70 pig-tailed macaques (*Macaca leonina*) from Khao Yai National Park. The animals were

Discovery of and Risk Characterization for a Novel Coronavirus at a Bat Guano Mining Site

Bat guano is sold for use as fertilizer for agricultural crops, including rice and orchard fruit. A large amount of guano is harvested from a bat cave in Khao Chong Phran (KCP), a non-hunting area and PREDICT field surveillance site in Ratchaburi Province. Bat guano was collected and screened for priority viral families – under a collaborative project supported by PREDICT and the Thailand Research Fund. Several bat guano specimens were positive for a novel betacoronavirus (same group as Severe Acute Respiratory Syndrome (SARS-CoV) and MERS-CoV; Figure 1; Wacharapluesadee et al. 2013).

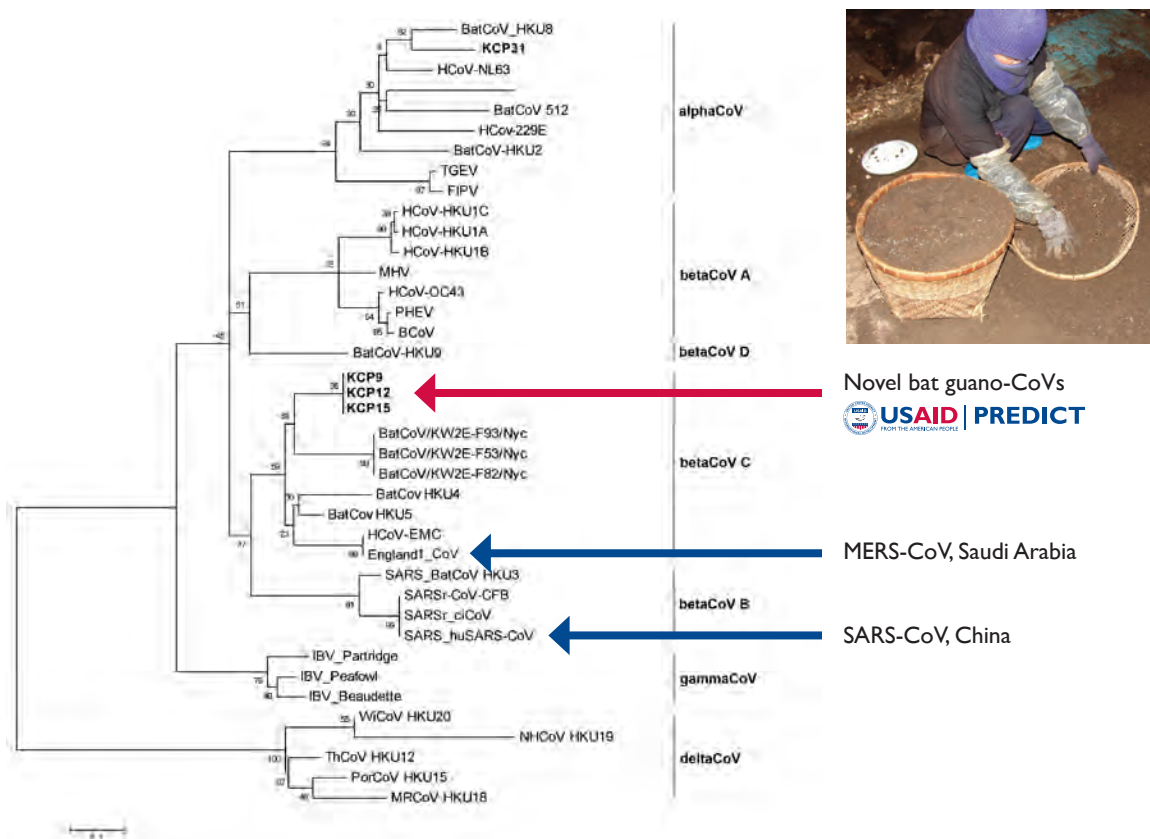


Figure 1. Phylogenetic tree showing 3 novel coronaviruses (CoVs; KCP9, KCP12, KCP15) detected in bat guano collected for fertilizer in Ratchaburi Province. Additional human and animal CoVs from the National Center for Biotechnology Information database are also included. Photo shows bat guano miner collecting guano at Ratchaburi cave site where the coronavirus was found.

Adapted from Wacharapluesadee et al 2013.

PREDICT staff worked with managers of the KCP non-hunting area and monks overseeing the temple and cave to better understand and characterize the risk of viral exposure to guano miners. PREDICT also worked closely with and advised EPT PREVENT on design of a more in-depth questionnaire to characterize the risk of human exposure and assessment of interventions to decrease the risk to miners.

CAPACITY BUILDING

The PREDICT Thailand team provided extensive training on animal handling, sample collection, and biosafety. In total, 171 personnel were trained under PREDICT activities. Over one third (37%, n=63) of the trainees were female professionals, including early career researchers and veterinarians. Training consisted of multiple hands-on, in-service training workshops, including human safety during animal capture and wildlife surveillance of EIDs for local DNP staff, best practices in sample collection and laboratory safety for hospital staff, regional bat ecology, and camel sample collection and laboratory training for MERS-CoV preparedness for staff of DNP, Zoo Park Organization of Thailand, National Institute of Animal Health, and Mahidol University.

PREDICT implemented broadly reactive consensus (genus/family level) PCR assays in the partner laboratory in Thailand to detect zoonotic and novel viruses and applied the PREDICT PCR protocols to pathogen screening for patients admitted to the Chulalongkorn Hospital.



Field surveillance activities in Thailand, including bat capture at various sites and macaque sampling.

SURVEILLANCE

Initially, surveillance in Thailand was focused on pathogens commonly detected in bats. The approach was expanded to include active surveillance for novel pathogens in a variety of wildlife hosts, in particular those taxa groups that pose a threat to humans or domestic animals (i.e. bats, rodents, and nonhuman primates). Surveillance activities were performed at eight sites in the eastern, northern, and southern parts of Thailand where there were high levels of contact with people through ecotourism or temple sites and where bats, rodents, and nonhuman primates all overlapped in their distribution (Figure 2). Additionally, we selected sites to maximize coverage across different parts of the country, including at the border with pristine and rapidly changing areas in Myanmar (Northwest), sites in close proximity to the border in Cambodia (East), and island sites in Southern Thailand.

Samples were collected from a total of 785 animals at eight surveillance sites in Thailand (Figure 3). Bats, rodents, and nonhuman primates were sampled at sites with high-risk disease transmission interfaces, including ecotourism areas, temples, bat guano mining sites, and national parks when human-wildlife conflicts were reported (e.g. macaques at campgrounds; Table 1).

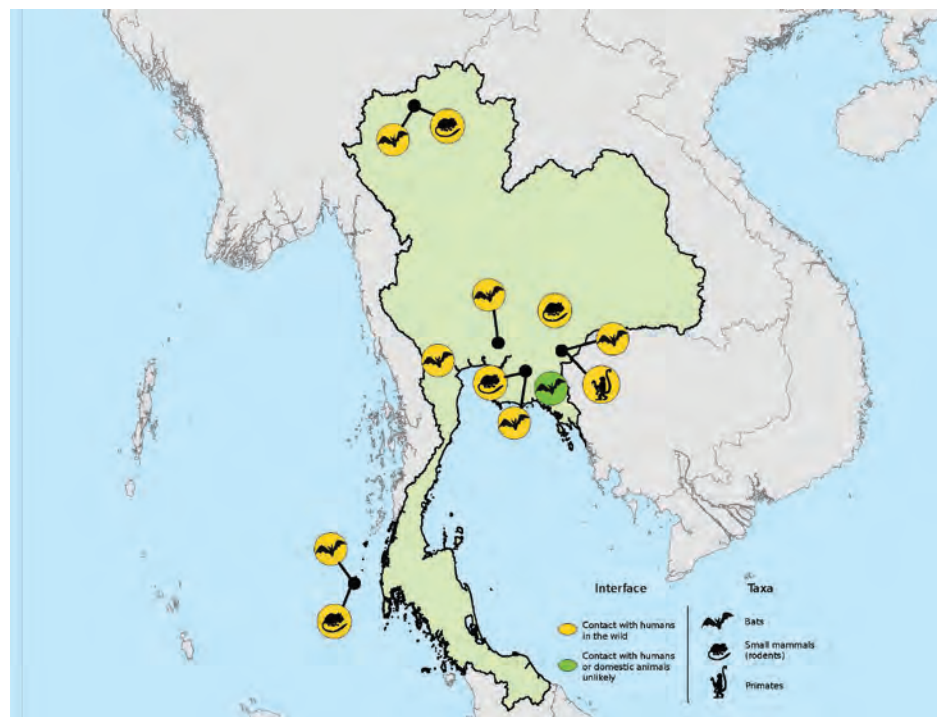


Figure 2. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

During the 2013 outbreaks of H7N9 influenza virus in China and MERS-CoV in the Middle East, PREDICT supported the Thai government through development of PCR protocols for

testing. In addition, as part of the National Laboratory Network, PREDICT was prepared to support the testing. In support, PREDICT led a two-day workshop and hands-on training for MERS-CoV preparedness to support livestock surveillance and laboratory diagnostics.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats
Ecotourism and recreational activities	120	4	3
In or near human dwellings	0	0	33
Other high-risk interfaces	0	4	328
Wildlife being studied	0	4	114
Protected areas	0	64	111
Total:	120	76	589

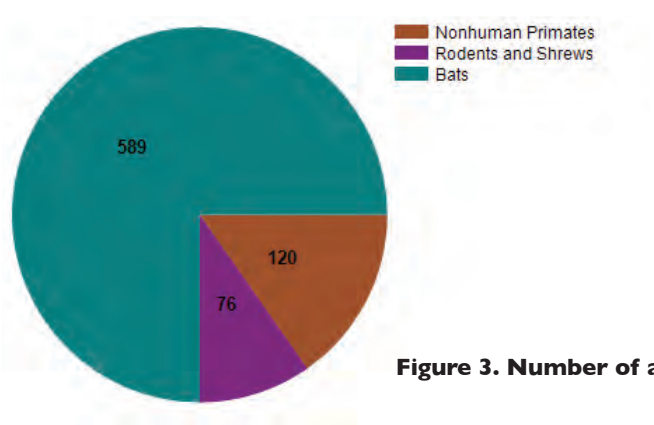


Figure 3. Number of animals sampled by taxa.

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In Uganda, parks provide protection for thriving populations of wildlife, all of which are surrounded by intensive animal agriculture and dense human populations.

PHOTOS BY GORILLA DOCTORS (LEFT) AND CHRISTINE JOHNSON (RIGHT)

UGANDA

Uganda is a highly populous nation in east-central Africa with a long-standing and well-established network of national parks. These parks provide protection for thriving populations of wildlife, all of which are surrounded by intensive animal agriculture and dense human communities, resulting in significant wildlife-livestock-human interactions. There are very few “buffer zones” between parks and land cultivated for crops or animals, which means that biodiversity is highly influenced by human use of landscapes. For example, Uganda is home to nearly half the world’s population of mountain gorillas, for which there is a thriving ecotourism industry that brings thousands of local and foreign people into contact with human-habituated gorillas (Gorilla Doctors 2014). Wildlife, including mountain gorillas, exit protected areas to forage in nearby cultivated fields, thereby coming into direct and indirect contact with people and domestic animals at the park margins. Additionally, a burgeoning cave tourism industry in Uganda brings local people and tourists into close contact with multiple species of bats.

Uganda has experienced several high-profile outbreaks of emerging infectious diseases (EIDs) in recent years, and intensive surveillance and research on the ecology of some of the more high-profile EIDs, such as those caused by filoviruses (e.g. Ebola virus, Marburg, and Bundibugyo; Polonsky et al. 2014), have rightfully focused the world’s attention on Uganda as a global “hotspot” for disease emergence. These factors, coupled with the infrastructure and efforts by multiple institutions and organizations (e.g. Uganda Wildlife Authority, Makerere University College of Veterinary Medicine, Gorilla Doctors) in disease monitoring and surveillance have facilitated the development and implementation of a comprehensive wildlife zoonoses surveillance program by PREDICT in Uganda.

FOR A SUMMARY OF THIS SECTION GO TO WWW.UGANDA.PREDICT.GLOBAL

Uganda's strong foundation of academic and public health networks with partnerships throughout the region have great potential for successful application of a One Health approach to emerging pandemic threats in Uganda and throughout the East Congo Basin. The PREDICT project in Uganda further improved capacity to conduct wildlife disease surveillance in order to better characterize zoonotic disease risks and to support the Ugandan government in its disease outbreak response and preparedness efforts.

PARTNERS

PREDICT-Uganda was implemented by Gorilla Doctors, a partnership between the non-governmental organization Mountain Gorilla Veterinary Project (MGVP, Inc.) and the University of California, Davis. Key collaborating partners included Makerere University College of Veterinary Medicine, USAID, Animal Resources and Bio-security (COVAB) and the Uganda Wildlife Authority (UWA).

Makerere University's COVAB is Uganda's premiere academic institution of veterinary science and teaching and service delivery and is a semi-autonomous Institution of Higher Learning supported by the Government of Uganda. The mission of COVAB is to provide innovative teaching, learning, research, and services responsive to national and global needs. MGVP, Inc. is co-located within the COVAB complex. COVAB houses the Makerere University Walter Reed Project's fully-equipped and staffed influenza research laboratory that conducts routine avian influenza surveillance and diagnostics in Uganda, and with which MGVP, Inc. collaborated on laboratory diagnostics support and services for PREDICT.

The Uganda Wildlife Authority (UWA), created in 1996, is the official government body charged with management of the wildlife resources of Uganda. UWA's Veterinary Unit has the formidable charge of health management for all wildlife, including the mountain gorillas. As a flagship species, and the cornerstone of Uganda's ecotourism industry, the endangered mountain gorilla features prominently in the activities of UWA and its Veterinary Unit. UWA recognizes that collaboration and cooperation are needed to effectively manage and protect wildlife.

Other important partners in implementation of PREDICT activities in Uganda include: The Ngamba Island Chimpanzee Sanctuary and Wildlife Conservation Trust (CSWCT), Uganda Wildlife Education Center (UWEC), Budongo Forest Conservation Project (BFCP), and the Uganda Wildlife Veterinary Network (UWVN).

MAJOR ACHIEVEMENTS

- Sampled 1,307 wild animals, including 739 nonhuman primates, 142 bats, 365 rodents, and 61 animals of other wildlife taxa.
- Supported the Government of Uganda National Task Force in several disease outbreak investigations, including Ebola virus disease and yellow fever: PREDICT led the wildlife surveillance efforts for these investigations to better understand the role that wildlife may have played in the outbreaks and to identify best strategies for effective prevention, preparedness, and response (see Success Story for more information).

PREDICT collected samples from several bats found dead around human dwellings in Mbunga, outside Kampala.



PHOTO BY GORILLA DOCTORS



Makerere University veterinary and wildlife students during a PREDICT training on proper personal protective equipment use at a field station in Lake Mburo National Park, Uganda.

- Installed essential laboratory equipment, supplies, and protocols at Makerere University to enable broad-based testing of hundreds of wildlife samples for viruses of pandemic potential; provided laboratory diagnostic support to other east-central African countries that were conducting disease surveillance in wildlife in order to strengthen diagnostic laboratory capacity regionally.
- Trained 36 wildlife veterinarians and 42 veterinary students in the principles and practice of safe and effective wildlife surveillance.
- Improved facilities and acquired equipment for the safe and secure transport and storage of biological samples collected from wildlife.

- Conducted systematic sampling of wildlife across a land-use gradient in the Bwindi-Mgahinga Conservation Area (as part of the “Deep Forest study”), which was designed to test theories regarding how landscape disturbance influences the emergence of wildlife pathogens in people.
- Introduced a novel, cell phone-based system that assists the Uganda Wildlife Authority with reporting on animal morbidity and mortality events in the Queen Elizabeth Conservation Area.

SUCCESS STORIES

Establishment of One Health Approach to Outbreak Response

PREDICT 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 prevention, preparedness, and response for the future. PREDICT-Uganda played an integral role in three important disease outbreak investigations, including Ebola virus and yellow fever (see below for information on disease outbreak response and preparedness), prioritizing sites for wild animal sampling activities in the field and collecting epidemiologic data on potential human risk factors, such as recent movements, hunting activities, and contact with and consumption of sick animals. The National Task Force now applies a One Health approach to disease outbreak investigation, control, and prevention in Uganda by incorporating wildlife investigations into disease outbreak response planning.

PREDICT veterinarians and staff of the Uganda Wildlife Education Center collecting samples from captive chimpanzees at UWEC during a routine health check.



Advancement of Wildlife Disease Surveillance and Diagnostics

PREDICT worked closely with the Uganda Wildlife Authority, Makerere University, and the Makerere University Walter Reed Project (MUWRP) to improve infrastructure and systems for detecting pathogens in wildlife. Specifically, PREDICT piloted a cell phone-based monitoring system for reporting wildlife mortality events, which is now integrated into UWA's ranger-based surveillance program in Queen Elizabeth Conservation Area. In addition, PREDICT established facilities for wildlife disease surveillance at COVAB and equipped MUWRP with equipment and protocols that enabled the laboratory to safely handle and test hundreds of samples from wildlife. Because the MUWRP laboratory is closely partnered with the Uganda Virus Research Institute (UVRI), human and wildlife disease investigations are now better linked as a result of the PREDICT effort.

CAPACITY BUILDING

Diagnostic Laboratory Capacity

PREDICT installed laboratory equipment and provided support and supplies for applying advanced molecular techniques to wildlife samples at the Makerere University Walter Reed Project (MUWRP) Influenza Research Laboratory. PREDICT implemented diagnostic testing protocols for nucleic acid extraction, amplification by polymerase chain reaction, and genetic analysis for viral genera/families known to cause illness, epidemics, and pandemics in people. Sequencing of positive PCR products and characterization of previously unreported viruses was performed at the University of California Davis and the Mailman School of Public Health, Columbia University, New York, both institutions located in the USA.

Training

PREDICT-Uganda personnel were trained on biosafety and PPE use; safe wildlife capture and handling; rodent, bat, nonhuman primate, and bushmeat sampling; sample handling, storage, packaging, and shipping; and laboratory safety. PREDICT provided the same training opportunities to 36 government and nongovernment veterinarians from Uganda Wildlife Authority, Makerere University Wildlife Department, Uganda Wildlife Education Center, Budongo Forest Conservation Project, Kibale Eco-health Project, and to 42 veterinary students at the Makerere University Wildlife Department, with a focus on basic principles of safe wildlife surveillance; personal protection; wildlife sampling; and biological sample collection, transportation, and preservation.

SURVEILLANCE

PREDICT sampled 1,307 free-ranging, captive, confiscated, and depredated wildlife (739 nonhuman primates, 365 rodents, 142 bats, and

PHOTO BY GORILLA DOCTORS



PREDICT Country Coordinator Dr. Benard Ssebide (left), PREDICT field veterinarian Dr. Racheal Mbabazi (standing), and Uganda Wildlife Authority rangers collecting samples from a kob exhibiting signs of illness in Queen Elizabeth National Park. This kob was diagnosed with bovine tuberculosis at postmortem, which was confirmed by culture and PCR.

61 animals from other wildlife taxa at high-risk disease transmission interfaces across Uganda, in places where wildlife are likely to have significant interactions with domestic animals and humans (Figures 1 and 2). Surveillance efforts were focused on primates, bats, and rodents; however, birds, carnivores, ungulates and domestic livestock were also sampled when relevant to specific interfaces and disease investigation efforts.

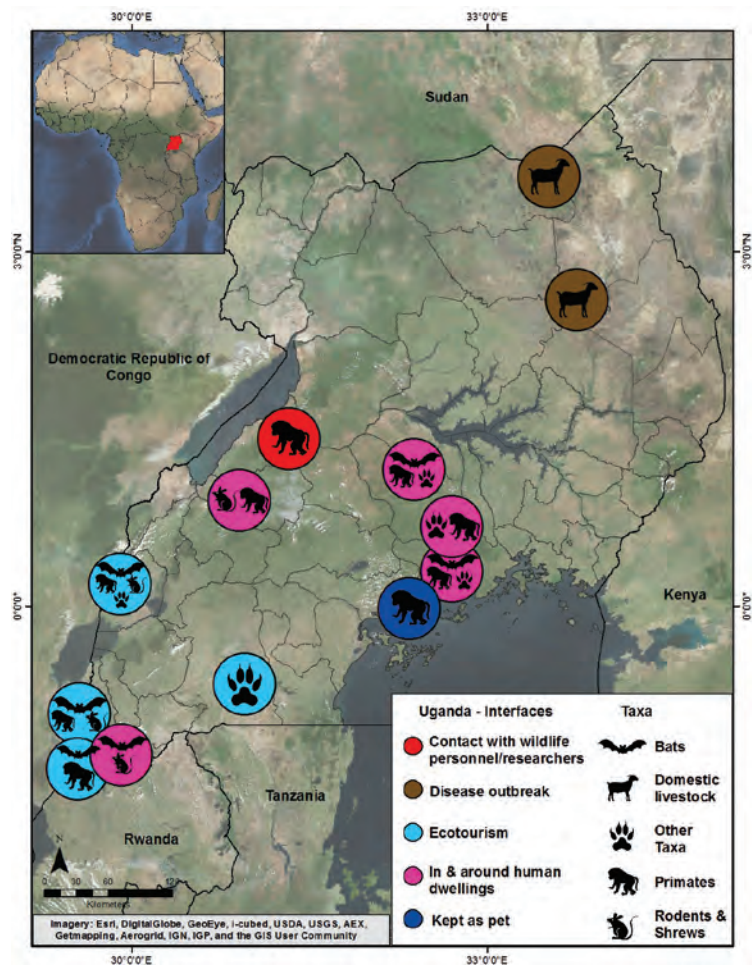


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

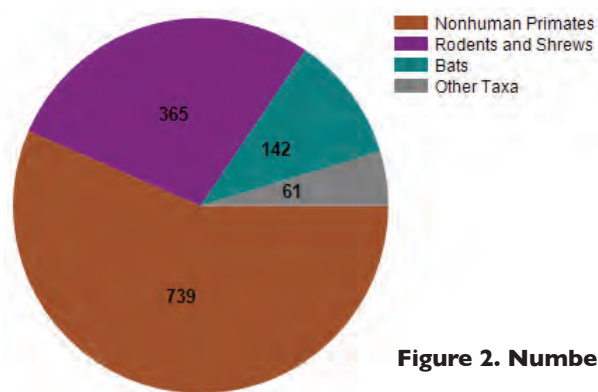


Figure 2. Number of animals sampled by taxa.

Surveillance was targeted at high-risk disease transmission interfaces where people and/or domestic animals come into direct or indirect contact with wildlife as a result of: ecotourism; presence in and around human dwellings and agricultural fields; the wildlife trade; human migration and travel; and increased exploitation of natural resources in areas that were previously uninhabited or very sparsely populated by human communities or where subsistence hunting and gathering activities were conducted in increasingly disturbed habitats (Table 1).

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
Agricultural settings	4	0	0	1
Ecotourism and recreational activities	330	42	53	4
In or near human dwellings	26	173	76	9
Hunted wildlife	2	13	0	21
Wildlife preying on livestock or their food	2	0	1	1
Pristine habitat	10	136	4	0
Wildlife trade	30	0	0	0
Wildlife being studied	9	0	0	0
Protected areas	219	1	8	0
Zoos and sanctuaries	107	0	0	0
Other high-risk interfaces	0	0	0	25
Total:	739	365	142	61

Animal Mortality Monitoring Program (AMMP)

PREDICT piloted a novel cell phone-based animal mortality monitoring program in Queen Elizabeth Conservation Area in collaboration with the Uganda Wildlife Authority. The pilot study was implemented by the Smithsonian Institution in collaboration with UWA and MGVP, Inc.. The aim of the program was to serve as an early warning system for emerging infectious diseases that can pass from animal populations into the human population. Rangers in Queen Elizabeth Conservation Area used Java enabled phones (with GPS functions) during their daily patrol to report any dead or sick wild animals. Authorities were alerted to reports of wildlife morbidity and mortality in real time through the mobile phone based internet technology.

Over 1695 records of sick (n = 831) and dead (n = 864) animals of more than 30 species were reported using this system. Among these reports, 145 cases were identified by rangers as potentially disease-related. Starting in January 2014, a team of PREDICT and UWA veterinarians and researchers responded to reports and collected samples from specific cases for pathogen detection, providing training, supplies, and support to the Pathology Department at Makerere University. Specifically, UWA and the AMMP team responded to 50 field reports of wildlife mortalities and collected samples from 10 cases: eight of these mortalities have been investigated by veterinary pathologists at the time of this publication, and causes of death

included infectious disease, trauma, and suspected toxicity. The mortalities were in proximity to human inhabited areas, and several of the carcasses were butchered and consumed by people in local communities, highlighting the potential for cross-species transmission of pathogens through the shared environment or through direct contact and consumption. The pilot program illustrated that: 1) animal mortality events can be reported in real-time; 2) the cell phone-based methodology is a simple, fast, effective, easily replicable, and inexpensive means to report morbidity and mortality; 3) sample retrieval for laboratory diagnostics is feasible; and 4) when implemented in conjunction with a pathology program, can be an effective means to document and monitor mortality and potential pathogenicity of pathogens in animal hosts.

Deep Forest Study

Land-use change represents one of the most substantial and pervasive threats to biodiversity. Furthermore, land-use change is an important driver of novel disease and zoonotic disease emergence events, as it modifies the risk of cross-species transmission by perturbing the dynamics of pathogens in wildlife hosts and/or by bringing new pathogens and hosts into contact. PREDICT tested theories regarding how wildlife pathogens emerge to infect people by conducting systematic sampling of wildlife across a gradient of human land use in the Bwindi-Mgahinga Conservation Area as part of the global PREDICT Deep Forest study (see Deep Forest section), which was also conducted in Brazil and Malaysia. The aim of the Deep Forest study was to evaluate how increasing land-use development affects: 1) patterns of biodiversity; 2) corresponding patterns of viral diversity; and 3) patterns of human occupancy, abundance, and behavior that may influence contact rates with wildlife in dynamic landscapes.

In Uganda, for the Deep Forest study, we systematically sampled wildlife at eight field sites situated along a gradient from a pristine forest landscape to a semi-disturbed landscape to a highly disturbed, semi-urban landscape. Disturbance was measured at two scales: 1) the landscape scale, calculated from satellite imagery, and 2) the local (site) scale, calculated from transect surveys. Deep Forest biodiversity sampling was focused on three high-risk mammal groups: rodents, bats, and non-human primates. At each site, standardized wildlife surveys were used to characterize local species richness and diversity. From each animal captured within these

surveys, blood, saliva, and fecal swab samples were collected, with urine and feces also opportunistically collected.

Each sample was analyzed in the laboratory by consensus PCR for 15 high-risk viral genera/families of interest. A subset of the samples was also analyzed with metagenomic deep sequencing, which allowed the detection of the entire community of viruses within the samples. Field surveys were conducted twice per year at each site (once in the wet season and once in the dry season) to minimize the effect that seasonality might have on the likelihood of detection of host and virus species.

A rodent trapped in Bwindi Impenetrable National Park for the PREDICT Deep Forest study.



PHOTO BY GORILLA DOCTORS

Concurrent with the wildlife sampling, the Deep Forest Human Contact (DFHC) survey was deployed to characterize human-animal contact at each study site. A total of 200 households situated in or immediately adjacent to study sites were surveyed. Particular attention was paid to types and frequencies of contact with bats, rodents, and non-human primates, as well as with other types of wild and domestic animals. The majority of households reported rodents and primates in or around their dwellings, with more than half reporting being bitten or scratched by wildlife (primarily rodents); additionally, some respondents reported that they had eaten wild animal meat in the past.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT assisted the Uganda National Task Force in disease outbreak response during the following outbreak investigations:

Yellow Fever Outbreak in Northern Uganda (November 2010)

In November 2010, the Ministry of Health received reports of an undiagnosed illness in northern Uganda. A team of Ministry of Health staff obtained samples from the human cases for testing. Preliminary results from laboratory investigations conducted by the Uganda Virus Research Institute (UVRI), Central Public Health Laboratories (CPHL), and eventually CDC Atlanta were negative for Ebola, Marburg, Crimean-Congo hemorrhagic fever, Rift Valley fever, and bacterial pathogens, such as typhoid.

In response to the need for additional epidemiological and laboratory investigations, the Ministry of Health in collaboration with the Ministry of Agriculture Animal Industry and Fisheries (MAAIF), Makerere University College of Veterinary Medicine, School of Public Health Makerere, WHO, USAID, African Field Epidemiology Network (AFENET), and CDC sent multi-sectoral transdisciplinary teams comprised of physicians, epidemiologists, veterinarians, and environmental health officers to facilitate the extended investigations and initiation of preliminary control measures. The PREDICT team was asked to participate on both the Epidemiology and Surveillance and the Veterinary and Vector subcommittees, providing technical expertise in the area of wildlife surveillance.

The objectives of the investigations were to: 1) conduct extended epidemiological and laboratory investigations to enable adequate description and characterization of the outbreaks; 2) establish systems for case identification, referral, isolation, infection control, and clinical management; 3) conduct an environmental risk assessment to determine the role of environmental exposures in the transmission of the illness under investigation; 4) investigate reports on the ongoing epizootic and determine its zoonotic potential; 5) assess high risk behaviors and knowledge gaps to facilitate development of an appropriate risk communication strategy; and 6) ensure effective coordination of the investigation and response efforts.

Each of the specialized teams conducted community visits to identify cases; interview the cases and/or relatives of the deceased cases; and collect human, environmental, and animal samples. PREDICT used available human epidemiological and clinical case information and worked closely with the CDC officers on-site to prioritize sites for animal sampling activities in the field, including visits to case homes to gather additional epidemiologic information ranging from peoples' observations and consumption of sick animals, recent movements, involvement in wildlife hunting, and bush meat consumption. In addition, PREDICT sampled animals, primarily rodents and domestic livestock, near homes and villages of affected people. Bushmeat

hunters agreed to cooperate with PREDICT and bring back carcasses for sampling; however, no hunters had successful hunts while the PREDICT team was in the field. The outbreak was eventually confirmed by the CDC as yellow fever in late December.

Ebola Virus Disease Outbreak in Luwero (May 2011)

In May 2011, a patient with signs of viral hemorrhagic fever presented to a hospital in the Luwero District and died. Ebola was confirmed through testing performed by UVRI. The Ministry of Health in Uganda established a National Task Force responsible for the outbreak investigation and response. PREDICT assisted the National Task Force through wildlife surveillance efforts aimed at identifying the potential source of the outbreak.

The wildlife surveillance activities were implemented in collaboration with UWA, MAAIF, Ministry of Health, CDC Uganda, and AFENET. PREDICT provided the materials and expertise needed for capture and sampling of potential wildlife hosts, the associated field investigations, and the laboratory analyses conducted at UVRI, MAAIF, and Columbia University. PREDICT sampled 10 vervet monkeys, two bush pigs, and one bushbaby. Viral pathogen testing for arenaviruses, flaviviruses, filoviruses, paramyxoviruses, and coronaviruses was performed, and all samples tested negative.

Ebola Virus Disease outbreak in Kibale (July 2012)

In July 2012, Ugandan health officials and WHO reported an outbreak of Ebola virus disease in the Kibale District, Uganda. This was confirmed by laboratory investigations conducted at UVRI, and the virus strain was identified as Sudan virus. The suspected index patient was a 16 year-old female. The Ebola National Task Force established subcommittees to implement outbreak response activities, including an Ecological Studies (ES) subcommittee to initiate and coordinate ecological surveillance in and around the outbreak area.

The ES subcommittee was tasked with: 1) determining if there are any links between the index case and possible animal reservoirs, e.g. monkeys, bats, and other animals; 2) identifying wild animal species that may be natural reservoirs for Ebola virus in the Kibale district; 3) collecting

samples to screen for Ebola virus which may be circulating in these wild and domestic animals as reservoir species or as a result of primary or secondary spillover; and 4) characterizing human-animal interactions that may increase opportunities and risk of contracting and transmitting the virus.

PREDICT conducted a human-wildlife contact survey in the areas surrounding the outbreak to characterize human-animal interactions that may have increased opportunities and the risk of contracting and transmitting the viral pathogens. Surveyed households (n=54) reported that rodents, bats, and primates were by far the most common wildlife species involved in human-wildlife

Bat trapping and sampling in Bwindi Impenetrable National Park for the PREDICT Deep Forest study.



PHOTO BY GORILLA DOCTORS

interactions. Most households reported seeing rodents, bats, or primates near their dwellings, with 78% of households reported seeing rodents inside their dwelling and 22% of households seeing both bats and rodents in their dwelling. Households also reported having direct contact with rodents in their home, mainly by being bitten by a live rodent or handling a dead rodent. Nearly half of the households also reported consuming fruit or vegetables that had evidence of being damaged or partially consumed by wild animals, most commonly by rodents, primates, and bats. Based on data obtained through this preliminary survey, there is sufficient contact between humans and bats, nonhuman primates, and rodents to enable transmission and spillover of pathogens commonly shared by these taxonomic groups.

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PHOTOS BY WILDLIFE CONSERVATION SOCIETY VIETNAM

Vietnam has capitalized on its geographic position to play a major role in wildlife trade in the region and is known as a trade hub for wildlife in Indochina.

VIETNAM

Vietnam is located in Southeast Asia and shares borders with China to the north and Lao PDR and Cambodia to the west. The country spans 330,951 km² (Vietnam General Statistics Office, Ministry of Planning and Investment), and as of 2012, the population of Vietnam was approximately 89 million (World Bank 2014). Vietnam is rich in biodiversity with approximately 1,534 known species of amphibians, birds, mammals, and reptiles (Mongabay 2006). However, many of Vietnam's iconic wildlife, like its endemic langur species, have been hunted close to extinction (Stone 2014). Human population growth and economic development have also driven large-scale land-use change with human encroachment into natural habitats putting additional pressure on wild species.

Vietnam has capitalized on its geographic position to play a major role in wildlife trade in the region and is known as a trade hub for wildlife in Indochina, with sources and destinations extending beyond Southeast Asia. A major component of the illegal wildlife trade thrives on the domestic and international demand for wildlife as food, targeting taxa from mammals to birds (WCS 2012). Commercial wildlife farming has also been developed in Vietnam and has rapidly expanded in numbers, species, and scale since 2000 when development of wildlife farms was encouraged by national action plans in Vietnam and supported by provincial directives (Thomson 2008). The expansion of wildlife farming has outpaced the establishment of strong enforcement of regulations and monitoring capacity of the authorities leading to reports of significant numbers of animal escapes, direct exploitation of the wild population to supplement farm stock, and poor provision of veterinary care (WCS 2008).

As a country heavily involved in the trade of wildlife (hunting, farming, and consuming wildlife locally; sourcing wildlife from neighboring countries; and trafficking wildlife across the region) and with a history of land-use change and population growth, the interfaces among wildlife,

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livestock, and humans in Vietnam are intense. PREDICT in Vietnam focused on conducting and building capacity for disease surveillance in wildlife at these high-intensity human-wildlife interfaces to detect pathogens of pandemic potential. The majority of surveillance was focused along the wildlife trade value chain, given the multiple opportunities for pathogen spillover, amplification, and spread. PREDICT, in collaboration with local authorities and research institutions, collected samples from wildlife for sale in markets, slaughtered for human consumption in restaurants, raised on wildlife farms, and confiscated by the authorities from the illegal wildlife trade. PREDICT worked with national laboratories and universities to develop and apply protocols for disease detection in these samples and to identify novel viral pathogens.

PARTNERS

PREDICT partners in Vietnam included the Wildlife Conservation Society (WCS), USAID, Department of Animal Health (DAH) of the Ministry of Agriculture and Rural Development (MARD), and the Hanoi University of Agriculture (HUA), now called the Vietnam National University of Agriculture.

Other local partners included:

Socialist Republic of Vietnam

- Regional Animal Health Office No. 6 (RAHO6)
- National Center for Veterinary Diagnostics (NCVD)
- Lam Dong Province Forest Protection Department (FPD)
- Dong Nai Province Forest Protection Department
- Soc Trang Province Sub-Department of Animal Health
- Dong Thap Province Sub-Department of Animal Health

Non-governmental research partners

- The Cat Ba Langur Conservation Project
- Animals Asia Foundation
- Carnivore and Pangolin Conservation Program

MAJOR ACHIEVEMENTS

- Advanced adoption of the One Health approach in Vietnam through a series of events and the establishment of a network to support Vietnam's One Health activities, including supporting Vietnam's participation in global One Health initiatives like the OIE Global Conference on Wildlife *"Animal health and biodiversity: Preparing for the future"*. These activities were convened to increase awareness of the importance of wildlife diseases and the risk that human behaviors at the wildlife-human interface contribute to increasing the transmission and emergence of zoonotic diseases (see Success Stories for more information).

An artificial roost at a bat guano farm in Soc Trang Province.



PHOTOS BY WILDLIFE CONSERVATION SOCIETY VIETNAM

- Initiated disease surveillance on wildlife farms in Vietnam through a collaborative effort between the DAH and the FPD in the pilot province of Dong Nai in southern Vietnam, which has one of the highest densities of wildlife farms in the country (see Success Stories for more information).
- Established capacity to safely conduct wildlife pathogen surveillance in Vietnam through extensive training on animal handling, sample collection, and biosafety. In total, 150 animal health officers received field-based training under PREDICT activities. Training included safe animal capture and handling, sample collection and storage, biosafety, and use of PPE.
- Raised awareness of the risk of disease spillover, amplification, and spread through wildlife trafficking by incorporating content on disease risks at the wildlife-human interface into training programs delivered to over 200 FPD and other enforcement officials in Vietnam.
- Established key laboratory capacity for virus detection at the HUA National Key Laboratory of Veterinary Biotechnology and the DAH RAHO6 Laboratory in Ho Chi Minh City. The two facilities provide national coverage for these services. Protocols for PCR screening for ten viral families (arenaviruses, flaviviruses, paramyxoviruses, hantaviruses, bunyaviruses, coronaviruses, henipaviruses, filoviruses, herpesviruses, and rhabdoviruses) were implemented with training also extended to the NCVD in Hanoi.

SUCCESS STORIES

Supporting the One Health Approach in Vietnam

PREDICT co-organized and supported a series of events in Vietnam that expanded the One Health network across the country and promoted a One Health approach to understanding and preventing the emergence of diseases of pandemic potential. The kick-off event held in March 2011 was a workshop entitled “*Wildlife and Emerging Infectious Diseases in Vietnam – Current Activities and Visions for the Future*” that attracted over 100 participants from across the country, representing the human health, animal health, and environmental sectors. The workshop, with its focus on wildlife health, was the first of its kind to be convened in Vietnam



PHOTO BY BORIPAT SIRARONRAT



PHOTO BY LAUREL FAIN, USAID

Photo left: Collecting samples from fruit bats in Soc Trang Province.

Photo right: Practicing wildlife sample collection methods during the Dong Nai Wildlife Health Training.



Participants at the Dong Nai Wildlife Health Training.

and facilitated the establishment of a network of human and animal health and wildlife professionals. The participants assessed prior and on-going research in the area of wildlife health and identified gaps in Vietnam's capacity to identify, investigate, diagnose, and respond to disease outbreaks with wildlife involvement. The gap analysis informed the development of PREDICT training activities and capacity building in the area of wildlife disease surveillance and pathogen detection in Vietnam. The gap analysis also guided the identification of areas of research and infectious disease challenges that would benefit from a One Health approach. The One Health network continues to be engaged in addressing some of the most critical challenges at the interface of wildlife, livestock, and human health in Vietnam as outlined through a few examples below.

PREDICT and network members contributed to the development of the "Vietnam Integrated National Operational Program on Avian Influenza, Pandemic Preparedness and Emerging Infectious Diseases (AIPED), 2011-2015" through a series of workshops and meetings organized by MARD, the Partnership for Avian and Human Influenza, and USAID. PREDICT co-organized the workshop entitled "Developing a 'One Health' approach for the period 2011-2015: addressing high impact infectious disease risks at the animal-human-environment interface" which was instrumental in establishing One Health as an important component of the Vietnam pandemic preparedness strategic plan, in particular highlighting the very important need for improved communication among the wildlife, livestock, and human health sectors.

PREDICT and network members provided support to PREVENT and other Emerging Pandemic Threat project partners around the topic of biosecurity on wildlife farms in Vietnam and participated in the "Expert Consultation Meeting: Strengthening Wildlife Farm Biosecurity and Supporting the Development of Good Production Practices" workshop in January 2013. As a direct follow-up to the workshop, PREDICT and network members joined the wildlife farming biosecurity and conservation subcommittee of the Vietnam Bio-Security Working Group, a collaborative effort between the Department of Livestock Production of MARD and the Food and Agriculture Organization of the United Nations (FAO) in Vietnam.

National 'One Health' conferences became biennial events in Vietnam with the 2013 event, which focused on the implementation of a One Health approach to infectious disease risks at the human-animal-ecosystem interface, held in Hanoi April 4-5, 2013. The One Health network in Vietnam

Common palm civet on a wildlife farm in Dong Nai Province.



PHOTOS BY WILDLIFE CONSERVATION SOCIETY VIETNAM



Slaughtering field rats in a wet-market in Dong Thap Province.

also kept in touch and informed through the distribution of PREDICT One Health Media Digest (digital media stories on wildlife, domestic livestock, and human disease outbreaks in Vietnam and internationally) which was launched in 2011. The recipients included the One Health network members, 94 individuals from government agencies, academic institutions, multi-lateral and donor organizations, NGOs, and other interested individuals.

PREDICT Initiates Disease Surveillance on Wildlife Farms in Vietnam

The DAH and the FPD joined forces to conduct disease surveillance on wildlife farms in Dong Nai Province in southern Vietnam from November 2013 through March 2014. Dong Nai Province borders Ho Chi Minh City and has one of the highest densities of wildlife farms in the country with over 1,000 farms raising reptiles, wild rodents, primates, birds, carnivores, and ungulates. The collaborative effort represented the first formal activity implemented jointly by the DAH and the FPD to address disease in wildlife. The joint surveillance effort was both an important example of operationalizing One Health in Vietnam and an important training opportunity as described below.

Before the launch of the surveillance effort, PREDICT-Vietnam held a series of meetings with Dong Nai Province officials to develop a field-based training program on wildlife diseases and conduct biosecurity risk assessments for wildlife and veterinary practitioners in the province to prepare them for the planned wildlife farm disease surveillance effort.

The wildlife health training was held in November 2013 at the launch of the wildlife farm surveillance effort and was co-organized and supported by PREDICT and RESPOND. The training covered wildlife disease diagnosis and management, handling of samples from wildlife for pathogen detection, the use of personal protective equipment (PPE), and biosecurity risk assessments on farms. Training participants, 38 in total, included rangers and officials from the Dong Nai FPD, veterinarians and animal health officers from the Dong Nai sub-DAH, and wildlife farm

owners. Trainers included lecturers from the Vietnam One Health University Network (VOHUN), PREDICT, and RESPOND staff. The field-based component of the training included conducting biosecurity assessments and collecting samples from wildlife species on three farms and one Department of Agriculture and Rural Development (DARD) rescue center. A total of 162 samples (primarily feces and environmental samples) were collected from seven different species of wildlife, including the common palm civet, Malayan porcupine, wild pig, pig-tailed macaque, crab-eating macaque, stump-tailed macaque, porcupine, civet, and bamboo rats.

As a direct follow-up to the November 2013 wildlife disease surveillance and wildlife farm biosecurity training, PREDICT worked with Dong Nai DARD in March 2014 to provide extensive field-based training on disease surveillance on wildlife farms to local district level rangers and veterinarians. Local veterinarians and forestry rangers joined the PREDICT team to collect samples from wildlife farms within their district's jurisdictional boundaries, visiting on average three farms per district, and collecting approximately 100 samples per district. The sampling was focused on PREDICT priority species, including bamboo rats, porcupines, and

nonhuman primates. Some sampling of civets was performed at the request of DARD. Overall PREDICT and DARD staff covered 11 districts, collecting samples from 539 individuals. Training participants included 13 DARD Forestry Protection Officers (one female and 12 male) and nine Veterinary Officers (one female and eight male). All of the local veterinarians and forestry rangers had participated in the November 2013 introductory training held in Dong Nai Province center. The March 2013 field-based training gave participants the opportunity to review and practice sampling methods (collection, labeling, storage, and transport), use of PPE, and the collection of saliva samples from nonhuman primates. It also reinforced these critical skills for officers on the front lines of wildlife disease surveillance and outbreak response.

CAPACITY BUILDING

Wildlife Disease Surveillance

PREDICT wildlife disease surveillance capacity building activities in Vietnam focused on building the field-based skills required for wildlife identification, safe handling, and sample collection for pathogen detection. PREDICT provided field-based training in wildlife disease surveillance for provincial-level and district-level animal health officers in the Mekong Delta region of Vietnam through their participation in sampling events in markets, restaurants, bat guano farms, and religious sites in the region. Veterinary students from HUA were trained in collection of a variety of specimen types from wildlife and environmental samples in Hanoi area restaurants, as well as the correct use of PPE. In addition, non-invasive techniques for the collection of saliva samples from nonhuman primates were taught to a range of animal health and wildlife managers in the north and south of the country.

PREDICT collaborated with VOHUN and RESPOND to train provincial-level and district-level veterinary and wildlife/forestry officers to conduct disease surveillance and biosecurity risk assessments on wildlife farms in Dong Nai Province in southern Vietnam, and a range of professionals working in wildlife rescue centers across the country were trained to collect samples from wildlife for pathogen detection. In the early stages of PREDICT work in Vietnam, the team focused on wildlife species identification training with animal health officers and produced a poster focused on the identification of PREDICT priority species for surveillance.

Laboratory Diagnostics

PREDICT laboratory diagnostics capacity building in Vietnam focused on training in molecular diagnostics, laboratory biosafety, and optimizing viral screening protocols for wildlife samples, as well as the provision of additional equipment and needed supplies and reagents for in-country laboratories. PREDICT laboratory capacity building trainings in Vietnam were kicked off with a “Laboratory Biosafety for Wildlife Diagnostics” workshop co-hosted by HUA’s Faculty of Veterinary Medicine for 16 laboratory and faculty members from HUA and two laboratory staff each from NCVD, RAHO6 Laboratory, and Oxford University Clinical Research Unit (OUCRU), as well as one laboratory expert from FAO. The initial workshop was followed by an introductory and advanced training in PREDICT molecular diagnostics.

PREDICT molecular diagnostics trainings covered a wide range of new technologies for pathogen detection and new sample preservation methods. The use of appropriate sample storage equipment and protocols was emphasized, and the use of new laboratory equipment was covered. The PREDICT PCR positive control was introduced, and, as a result of this

series of trainings and additional one-on-one support, we successfully established capacity for screening for arenaviruses, flaviruses, paramyxoviruses, hantaviruses, bunyaviruses, coronaviruses, herpesviruses, henipaviruses, filoviruses, and rhabdoviruses. The HUA National Key Laboratory of Veterinary Biotechnology and the RAHO6 Laboratory in Ho Chi Minh City together now provide national coverage for these services. Training and the PREDICT PCR positive control were also extended to the NCVD in Hanoi.

Laboratory infrastructure for wildlife pathogen detection in Vietnam was supported by PREDICT with the provision of ultra-low temperature freezers, high speed centrifuges, and a range of sample processing equipment. In addition, rack systems and other equipment were installed to improve sample management and safe storage for the ultralow freezers placed in the HUA National Key Laboratory of Veterinary Biotechnology and the RAHO6 Laboratory in Ho Chi Minh City.

Wildlife Pathology Regional Training

PREDICT held the “First Regional Wildlife Pathology Workshop” in Asia in partnership with HUA and Smithsonian Institution. Twenty-five government and university pathologists (19 from Vietnam, three from Laos, and three from Cambodia) were trained in basic cytology, PPE use, necropsy skills, sample collection for disease investigation, introduction to histopathology, and advanced molecular diagnostics. Seventy-eight participants attended the morning theory sessions of the workshop. The workshop was hosted by the Faculty of Veterinary Medicine, HUA, with funding and training development provided by PREDICT.

Disease Risks Associated with Wildlife Trade

PREDICT collaborated with the WCS Vietnam Country Program to create and deliver a training module for wildlife law enforcement officials that introduced the One Health concept



PHOTOS BY WILDLIFE CONSERVATION SOCIETY VIETNAM

Photo left: Practicing DNA electrophoresis methods during the PREDICT Molecular Diagnostics Training at the Hanoi University of Agriculture.

Photo right: Practicing necropsy techniques during the PREDICT Wildlife Pathology Workshop at the Hanoi University of Agriculture.

and the human health risks associated with the wildlife trade, which is a significant wildlife-human interface in Vietnam. The module was presented at five wildlife law enforcement training workshops with government staff from the following departments and ministries in Vietnam: DAH, Department of Livestock Production, National Institute of Animal Husbandry, Vietnam Administration of Forestry, Forestry Directorate, and National Institute of Veterinary Research from MARD; Border Security Station from the Ministry of Defense; Vietnam Customs Department from the Ministry of Finance; Market Control Department from the Ministry of Industry and Trade; Environmental Police Department, Traffic Police Department, and Department of Agriculture Security from the Ministry of Public Security; Vietnam Environment Administration from the Ministry of Natural Resources and Environment; and People's Procuratorate of the Supreme People's Procuratorate.

SURVEILLANCE

Over 2,054 animals were sampled at high-risk disease transmission interfaces during 90 sampling events across Vietnam (Figure 1). Animals sampled included 1,288 rodents, 366 bats, 42 nonhuman primates, and 358 animals in other taxa (Figure 2).

Wildlife surveillance was conducted in partnership with the DAH and FPD of MARD and a number of non-governmental wildlife conservation organizations. High-risk disease transmission interfaces targeted for sampling included markets where live animals are sold, restaurants serving wild meat, wildlife farms, wildlife rescue centers/sanctuaries, and sites with wildlife present in and around human dwellings (Table 1). Sampling sites were distributed across Vietnam from the Mekong River Delta region in the south to sites in the north, including the national capital, Hanoi.

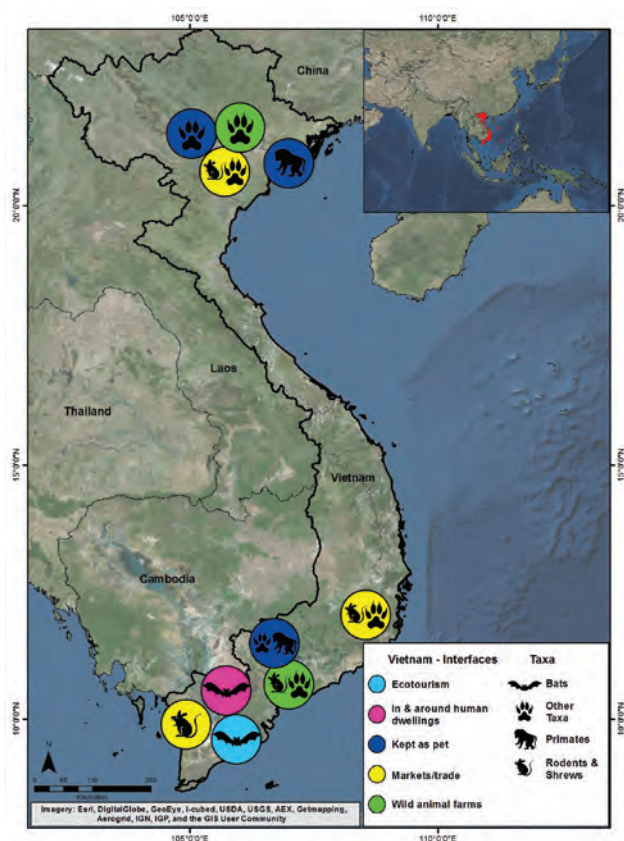


Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

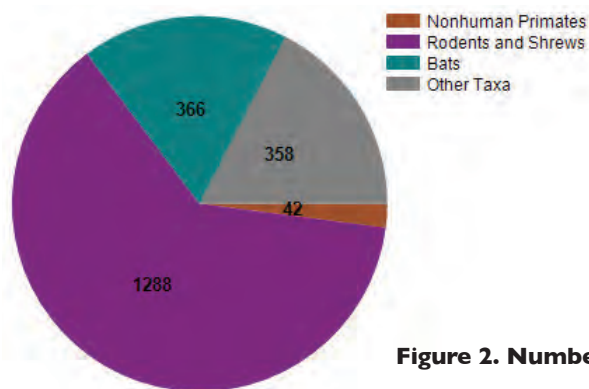


Figure 2. Number of animals sampled by taxa.

Table 1. Number of animals sampled according to targeted transmission interfaces.

Interface	Nonhuman Primates	Rodents and Shrews	Bats	Other Taxa
In or near human dwellings	0	0	366	0
Markets	0	372	0	0
Restaurants	0	298	0	132
Wildlife trade	0	189	0	0
Farmed wildlife	15	429	0	153
Zoos and sanctuaries	27	0	0	73
Total:	42	1288	366	358



PHOTO BY WILDLIFE CONSERVATION SOCIETY VIETNAM

A primate is chewing on a swab during a nonhuman primate sampling trip at Bao Son Zoo, Hanoi.

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT provided field-based training for forestry and veterinary officers on the “front lines” of disease outbreak events in wild and domestic animal populations in Vietnam. The capacity developed for wildlife surveillance and laboratory diagnostics is now available to apply to disease outbreak response.

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PREDICT CONSORTIUM MANAGEMENT



The PREDICT Consortium was a functionally collaborative and fully integrated working team that benefited from the experience of world leaders in zoonotic disease surveillance, epidemiology, disease ecology, and risk characterization.

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- Andrew Clements
- Murray Trostle
- Alisa Pereira
- August Pabst

LEAD CONSORTIUM INSTITUTIONS FOR PREDICT

- **UC Davis'** One Health Institute is active all over the world, working at the interface of animals, people, and the environment to solve complex problems that impact health and conservation.
- **EcoHealth Alliance** is the first group to identify bats as the reservoir of SARS-like coronaviruses, to define hotspots of emerging diseases, and identify drivers of disease emergence.
- **Metabiota, Inc.** (formerly known as Global Viral Forecasting, Inc.) has made seminal discoveries on the role of hunting of nonhuman primates and food handling in moving animal pathogens to humans.
- **Smithsonian Institution** and the National Zoo are among the founders of the field of conservation biology.
- **Wildlife Conservation Society** was the first conservation organization with a dedicated team of wildlife veterinarians deployed around the world, with programs focused on environmental stewardship and health problem-solving.

Other global partners include Columbia University; Boston Children's Hospital's HealthMap; International Society for Infectious Disease; and the University of California, San Francisco.

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Technical Director, William B Karesh, DVM, is the Executive Vice President for Health and Policy for EcoHealth Alliance, and serves as the President of the World Organisation for Animal Health (OIE) Working Group on Wildlife Diseases.

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Consisting of the Key Personnel and the most senior representative of the core consortium, the Executive Board shaped the management of all activities and ensured that all countries received the support needed to achieve the objectives according to the USAID approved work plans.

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- **Nathan Wolfe**, Metabiota (MB)
- **William Karesh**, EcoHealth Alliance (EHA)
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PREDICT TECHNICAL ADVISORY COMMITTEE

External guidance was provided to the PREDICT Team by an expert Technical Advisory Committee. In addition to assessing PREDICT progress, this group of distinguished scientists reviewed activities and plans and advised on priorities, strategic focus, and broader partner collaboration.

- **James Hughes**, Professor, Emory School of Public Health and former Director US CDC's National Center for Infectious Diseases
- **Pierre Formenty**, World Health Organization focal point and coordinator for Zoonotic Diseases and senior staff member of GOARN
- **Mo D. Salman**, Professor, Colorado State University College of Veterinary Medicine, extensive experience with building animal health epidemiology programs in severely challenged countries
- **C.J. Peters**, Professor, University of Texas Medical Branch, formerly of US Army Medical Research Institute of Infectious Diseases and Chief of CDC's Special Pathogen Unit
- **Lonnie King**, Dean, College of Veterinary Medicine, The Ohio State University, formerly Director of CDC's National Center for Zoonotic, Vector-Borne, and Enteric Diseases and previous Administrator USDA's Animal and Plant Health Inspection Service
- **James Childs**, Senior Research Scientist, Yale University, formerly Chief of CDC's Viral and Rickettsial Zoonoses branch and one of the world's experts on bat-borne and rodent-borne diseases
- **Donald Burke**, Dean, University of Pittsburgh School of Public Health, formerly of John Hopkins Bloomberg School of Public Health and US Walter Reed Army Institute of Research

