excerpted from
Reducing Pandemic Risk, Promoting Global Health

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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.
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.

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.
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.
To achieve these successes, 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 in laboratories operated by our in-country partners. Our efforts have resulted in more viruses detected in just five years than the total number of viruses previously recognized in mammals by the International Committee on Taxonomy of Viruses (ICTV; last version from ICTV in 2009 at beginning of PREDICT project). On the list of viruses detected thus far are many important human and animal pathogens, such as 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 Ebola viruses in humans during multiple EVD outbreaks in Africa. In fact, PREDICT has detected new coronaviruses numbering almost twice those previously acknowledged (both approved and proposed) by the ICTV by the end of 2009.

Analyses incorporating all virus detections validated our surveillance strategy – showing 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. 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 limited. Continuing use of our viral detection and discovery methods by in-country partners illustrates the establishment of long-term capacity for identifying known and novel viruses and should be increasingly useful in diagnosis of outbreaks for diseases of unknown origin, thus speeding up the detection of EID events.

PREDICT’s risk-based surveillance strategy was focused on situations where people have frequent, direct contact with wild animals. These high-risk disease transmission interfaces have occurred where diverse groups of wildlife viruses are available to infect susceptible humans or their domestic animals, as was seen with SARS emerging from wildlife markets, EVD and HIV/AIDS from butchering or handling of wild primates, and new strains of influenza in people

![Epidemiologic network map illustrating high-risk disease transmission interfaces for zoonotic viruses transmitted from wildlife to humans.](image-url)

**Figure 2.** Epidemiologic network map illustrating high-risk disease transmission interfaces for zoonotic viruses transmitted from wildlife to humans. High-risk interfaces are shown with node size proportionate to the number of viruses (red) reported for each transmission interface, categorized according to 1) direct contact with wildlife (blue); 2) indirect contact with wildlife (green); and 3) transmission by vector (yellow). From Johnson et al. 2014.
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.
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.
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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).

**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 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) will 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 Deep Forest 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.

REFERENCES
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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),
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 has 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 has also increased capacity within a laboratory network that has 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)
• 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.

• Intensively 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 has sampled 1,179 animals in Sabah since 2012.

• 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.

• PREDICT 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 has 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 includes 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

![Photo by Tom Hughes](image-url)
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 has received funding by the Ministry of Natural Resources and Environment to support ongoing wildlife surveillance activities. This development is 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.

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 is 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 is 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...
laboratory is 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 is also used for testing wildlife samples for PREDICT. VRI has been 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 has developed all standard operating procedures (SOPs) and protocols for WHGFL and is helping to improve and standardize protocols and SOPs for the three laboratories it has helped develop.

Training

To date, PREDICT has 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 has trained 21 DWNP officers from states on Peninsular Malaysia where PREDICT sampling has been conducted. The DWNP established a dedicated surveillance team to conduct routine surveillance and respond to outbreaks.
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 is not widely available in Malaysia, but a few institutions are 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 is standardized across the land-use gradient, and includes three different survey sites in each environment. Bats, rodents, and nonhuman primates are 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 have been 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.
Table 1. Number of animals sampled according to targeted transmission interfaces, Sabah.

<table>
<thead>
<tr>
<th>Interface</th>
<th>Nonhuman Primates</th>
<th>Rodents and Shrews</th>
<th>Bats</th>
<th>Other Taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural settings</td>
<td>14</td>
<td>181</td>
<td>254</td>
<td>22</td>
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<tr>
<td>Ecotourism and recreational activities</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>In or near human dwellings</td>
<td>5</td>
<td>26</td>
<td>30</td>
<td>2</td>
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<tr>
<td>Wildlife being studied</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Protected areas</td>
<td>71</td>
<td>64</td>
<td>450</td>
<td>15</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total:</td>
<td>129</td>
<td>271</td>
<td>735</td>
<td>44</td>
</tr>
</tbody>
</table>

Figure 3. Number of animals sampled by taxa, Sabah.

Figure 4. Number of animals sampled by taxa, Peninsular Malaysia.
Table 2. Number of animals sampled according to targeted transmission interfaces, Peninsular Malaysia

<table>
<thead>
<tr>
<th>Interface</th>
<th>Nonhuman Primates</th>
<th>Rodents and Shrews</th>
<th>Bats</th>
<th>Other Taxa</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Ecotourism and recreational activities</td>
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<td>In or near human dwellings</td>
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<tr>
<td>Wildlife trade</td>
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<td>0</td>
<td>190</td>
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<tr>
<td>Protected areas</td>
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<td>Zoos and sanctuaries</td>
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<td>14</td>
</tr>
<tr>
<td>Total:</td>
<td>945</td>
<td>128</td>
<td>176</td>
<td>237</td>
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</tbody>
</table>

**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 has 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.

**REFERENCES**


