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Reducing Pandemic Risk, Promoting Global Health

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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, “siloed” 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 karese
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.

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.

PHOTO BY TIERRA SMILEY EVANS

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.
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).

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.
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.

**REFERENCES**


