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Reducing Pandemic Risk, Promoting Global Health

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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).
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.](image)

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).
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).
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.

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*).

![Diagram](image)

**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
The *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 (Wacharapasuesadee 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](image_url)

*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 paraxmyxoviruses (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-Mundeke 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
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. 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, Adlhoch 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 (Adlhoch 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
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. Phylogenetis 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.
(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 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.
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 ZOONOTIC 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 cestodiases, posing a much greater threat to public health than other forms of taeniiasis 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 hepaviruses or homologs using large-scale PCR-based screening on samples from a range of mammalian species. In this study, non-primate hepavirus was found in three horses and was not associated with pathology (Lyons et al. 2012b). In previous studies, canine hepavirus 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 hepavirus). In addition, Lyons et al. (2014) screened large numbers of horses for viremia and for past exposure through serological assays for non-primate hepavirus and equine pegivirus. Approximately 43% of 328 horses were seropositive for non-primate hepavirus (three viremic), and 66% of horses were seropositive for equine pegivirus (12 viremic). Detection of hepavirus 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).

**PREDICT CITATIONS**

(includes PREDICT publications and publications that benefited from intellectual developments from the PREDICT project):


