excerpted from
Reducing Pandemic Risk, Promoting Global Health

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Mexico spans two biogeographic regions, the Nearctic and Neotropics, and ranks among the top ten countries for species richness (Martínez-Meyer et al. 2014). Its conical shape forces the convergence of major migratory corridors for many taxonomic groups, including mammals, birds, and reptiles. Due to its unique geological and climatic history, it represents less than 1% of the earth’s surface, yet holds 5-10% of the world’s biodiversity, including many endemic species (Mittermeier et al. 1997). Mexico’s natural wealth is also reflected by the great diversity of ecological processes, which have been altered dramatically by human activities in the past decades. Natural vegetation in Mexico continues to diminish; by 2002 it had been reduced to only 38% of its original area in the country (Sarukhán et al. 2009).

Anthropogenic impacts, such as land-use change, lead to perturbations of natural systems with potential effects on the likelihood of contact among wildlife, livestock, and people. Mexico is considered a hotspot for emerging infectious diseases (EIDs) of wildlife origin because of its high biodiversity and dense human population (Jones et al. 2008; Morse et al. 2012). Its potential as an EID hotspot has been illustrated by recent outbreaks of disease in people and wildlife, including pandemic H1N1 influenza virus, West Nile virus and other flaviviruses, coronaviruses, alphaviruses, and hantaviruses (Zepeda-Lopez et al. 2010; Anthony et al. 2013; Mann et al. 2013; Sotomayor-Bonilla et al. In Press). These events and their potential connection to land-use change and biodiversity have revealed the need to understand the relationships between pathogens, reservoirs (including people), and vectors (mosquitoes and ticks) in Mexico. Mexico’s unique biogeography and diversity make it an ideal location to investigate some of the most important questions regarding drivers of disease emergence.

FOR A SUMMARY OF THIS SECTION GO TO WWW.MEXICO.PREDICT.GLOBAL
PARTNERS
PREDICT partners in Mexico included EcoHealth Alliance (EHA), Facultad de Medicina Veterinaria y Zootécnica (FMVZ) and Instituto de Ecología, Universidad Nacional Autónoma de México (UNAM), USAID, and the Center for Infection and Immunity (CII) at Columbia University.

Other local partners included:

- Kalaan-Kab AC
- BIOCONCIENCIA
- Laboratorio de Medicina para la Conservación, Instituto Politécnico Nacional/Conservation Medicine Lab (IPN)
- Instituto Mexicano del Seguro Social (IMSS)
- Consejo Técnico Consultivo Nacional de Sanidad Animal/Technical Council of Animal Health (CONASA)
- Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación/Mexican Department of Agriculture (SAGARPA)
- Comisión Nacional de Salud Animal/Wildlife Health Commission
- Comisión Nacional de Áreas Naturales Protegidas/Commission for Protected Natural Areas (CONANP)
- Secretaría de Medio Ambiente y Recursos Naturales/Secretary of the Environment and Natural Resources (SEMARNAT)

MAJOR ACHIEVEMENTS

- Integrated research on human and animal systems to develop a One Health framework for the PREDICT project in Mexico (see Success Stories for more information).
- Met the major country goals for capacity building set by the Emerging Pandemic Threats program for PREDICT in the two-and-half-years that the project was active in Mexico.
- Detected novel viruses in bats, including novel herpesviruses, astroviruses, and coronaviruses.
- Trained 28 people (54% women) in core safety; animal capture, handling, and sampling skills; lab safety; PPE use and biosafety; zoonotic disease risks; disease modeling; animal care and use protocols; and lab diagnostic techniques.
- Collected samples from 2,028 animals, 99% of which came from high-risk taxa groups (i.e. bats and rodents).
- Provided PREDICT protocols, positive controls, and primers to laboratories in Mexico City (IMSS, UNAM, and IPN). All laboratories are now equipped and have the technical expertise to conduct family level viral diagnostics on clinical samples.
**SUCCESS STORY**

**Development of One Health Framework**

The PREDICT Mexico team integrated research on human and animal systems to develop a One Health framework for the program in Mexico. The team’s efforts resulted in two successful international conferences, several well-trained Master’s and PhD students, and the establishment of a wildlife health position on Mexico’s Technical Council of Animal Health.

**Initiating Discussions.** The International Conferences of Disease Ecology and Conservation Medicine were hosted by the PREDICT team along with Mexican universities and the non-profit Kalaan–Kab AC (Mexican Association of Conservation Medicine). The conferences, in 2011 and 2013, drew more than 150 attendees each from several organizations, including EcoHealth Alliance, UC Davis, Columbia University, the Smithsonian Institution, and the London Zoological Society. The conferences highlighted the importance of a One Health approach to zoonotic diseases and developing a national wildlife disease surveillance system.

PREDICT-Mexico also organized and participated in three symposia at the National Autonomous University of Mexico (UNAM). The meetings were held on different campuses, and the number of attendees ranged from 30 to 100. These symposia focused on the effect of anthropogenic activities, climate change, and biodiversity loss on disease dynamics, as well as new strategies to monitor wildlife health. One of the symposia also highlighted the role of women in science.

**Training Future Experts.** PREDICT-Mexico supported the training of several Master’s and PhD level students in disease ecology and evolution of disease at UNAM. Their research included pathogen discovery and the effect of species diversity and landscape change on the dynamics of infectious diseases.

One PhD student participated in a month-long exchange program to learn deep sequencing techniques at the Center for Infection and Immunity at Columbia University. The student’s study was published in the Journal of General Virology and described the discovery of 12 novel coronaviruses and one known coronavirus in bats from Mexico (Anthony, Ojeda-Flores et al. 2013). At the time of publication, one of the discovered viruses had a 95% amino acid identity to the coronavirus causing the Middle Eastern respiratory syndrome (MERS) outbreak in the Middle East. This finding informed on targeted surveillance for coronaviruses in bats living in areas with cases of MERS.

**Lasting Change.** The PREDICT team’s efforts in Mexico helped develop a One Health framework for emerging disease prevention and control on a national level. The team’s Country Coordinator was invited to present to three Mexican governmental departments. It was the first time Mexico’s health department (CONASA), agriculture department (SAGARPA), and health and natural resources department (SEMARNAT) came together to talk about connections between the health of wildlife and domestic animals. As a result of these efforts, a wildlife health position was established on the Technical Council of...
Animal Health, and the PREDICT Country Coordinator was invited to serve a two-year term as the World Organisation for Animal Health (OIE) representative.

Other contributions to the future of One Health platforms were assured through PREDICT’s significant contributions to higher education in Mexico. Our protocols were incorporated into the curriculum in the Disease Ecology and Conservation Medicine undergraduate course at the Veterinary School at UNAM. Furthermore, the PREDICT diagnostic laboratory protocols were implemented by other research faculty at the Veterinary School.

**CAPACITY BUILDING**

**Training**

Significant improvements in wildlife disease surveillance were made in Mexico as a result of PREDICT trainings on proper sample collection and management of data through the GAINS database. Thirty-two students and faculty members received training on the full set of protocols developed by PREDICT, including human and animal safety during wildlife capture, PPE, laboratory safety, sampling methodology, and zoonotic diseases. The team also trained 25 biologists in bat identification and more than 20 students on sampling of bats and rodents during a formal course at the Faculty of Medical and Veterinary Sciences, UNAM.

Following completion of the PREDICT project in Mexico, the local partners worked to ensure sustainability of in-country surveillance systems through efforts to implement a database similar to GAINS in Mexico that would integrate research from several Mexican universities. In addition, the partners developed a proposal with SEMARNAT to design a national database for epidemiological surveillance data for wildlife diseases.

**Laboratory**

PREDICT’s laboratory diagnostic protocols for detection of viruses of public and animal health importance improved the ability to screen for important viral genera/families, such as coronaviruses, paramyxoviruses, flaviviruses, arenaviruses, and hantaviruses. These protocols were implemented in at least three university laboratories and one national public health laboratory. The Mexican government now has the capacity to utilize the PREDICT protocols along with the universal controls for viral family-level PCR screening. The university laboratories that implemented PREDICT diagnostic protocols trained more than ten undergraduate and graduate students.

**SURVEILLANCE**

Initial disease surveillance sites were chosen based on a gradient of likely virus spillover from animals into humans. EID hotspot data from Jones et al. 2008 was utilized to select sites in Mexico City, Chiapas, and in the Calakmul Biosphere Reserve representing areas with potentially high, medium, and low likelihood of viral spillover.

Because theoretical models suggest that land-use change is a primary driver of emerging infectious diseases (Loh et al. In Review), PREDICT Mexico added additional sites across a human disturbance...
gradient from pristine forests to more disturbed urbanized locations. Surveillance was then focused in two areas in southeast Mexico with very high biodiversity. For each of the two study areas, the field team chose sites along a landscape disturbance gradient, with sites located in pristine, fragmented, and disturbed locations within the same ecoregion in order to support the disease hotspots theory (Jones et al. 2008) and investigate the relationship between land-use change and host and viral diversity.

The pristine sites were located in the Lacandona and Calakmul Biosphere Reserves (in Campeche and Chiapas Mexico, respectively). Intermediately disturbed sites with partial deforestation (i.e. fragmented) were selected within 10 km of the reserves, and highly disturbed (i.e. disturbed) sites were selected in the same ecoregion. This approach was later further developed using a landscape disturbance index for the Deep Forest study in Brazil, Malaysia, and Uganda. The diversity and richness of bats was estimated by habitat gradient to describe the effect of habitat loss on the assembly of bat communities. The data presented corresponds to 12 nights of sampling effort by habitat type. The diversity of each habitat was calculated with the Shannon’s index (H), and the richness (S) is the number of species in each habitat.

Surveillance was conducted at sites where there was potential for disease spillover from wildlife into people and one site where contact between wildlife and people was unlikely. The high-risk wildlife-human interfaces represented at the sites included wildlife in contact with park personnel/tourists or researchers, wildlife in contact with workers in extractive industries (e.g. logging, mining), and wildlife in or near human dwellings or agricultural fields (Figure 1; Table 1). Disease surveillance activities were focused on bats and rodents. A total of 2,028 animals were sampled (1,981 bats, 41 rodents, and 6 marsupials), representing 58 bat species and 11 rodent species (Figure 2).

Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.
Table 1. Number of animals sampled according to targeted transmission interfaces.

<table>
<thead>
<tr>
<th>Interface</th>
<th>Rodents and Shrews</th>
<th>Bats</th>
<th>Other Taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural settings</td>
<td>14</td>
<td>139</td>
<td>1</td>
</tr>
<tr>
<td>Ecotourism and recreational activities</td>
<td>0</td>
<td>270</td>
<td>0</td>
</tr>
<tr>
<td>Extractive industries</td>
<td>3</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>22</td>
<td>631</td>
<td>5</td>
</tr>
<tr>
<td>Wildlife being studied</td>
<td>0</td>
<td>129</td>
<td>0</td>
</tr>
<tr>
<td>Protected areas</td>
<td>2</td>
<td>759</td>
<td>0</td>
</tr>
<tr>
<td>Pristine habitat</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>41</strong></td>
<td><strong>1981</strong></td>
<td><strong>6</strong></td>
</tr>
</tbody>
</table>

**VIRUS DISCOVERY AND CHARACTERIZATION IN BATS**

Initially, consensus (genus/family level) PCR assays for 13 viral genera/families of health importance (coronaviruses, adenoviruses, herpesviruses, astroviruses, paramyxoviruses, filoviruses, bocaviruses, alphaviruses, flaviviruses, lyssaviruses, seadornaviruses, arenaviruses, and hantaviruses) were conducted on archived samples collected from 117 bats in the Mexican states of Chiapas, Oaxaca, and Tabasco. This work led to the discovery of novel viruses, including betaherpesviruses, gammaherpesviruses, coronaviruses, and astroviruses.

PREDICT also used unbiased high-throughput sequencing to uncover a highly diverse group of bat-derived viruses in bats from Africa, Mexico, and Central America that are related to hepaciviruses and pegiviruses within the family Flaviviridae. This testing identified seven new pegiviruses (*Flaviviridae*) in seven different species of bats (Quan et al. 2013). Evolutionary analyses indicate that all known hepaciviruses and pegiviruses, including those from humans and other primates, originate in bats (Quan et al. 2013). The biodiversity, phylogenetic divergence, and global distribution of the viruses suggest that bats are a natural reservoir for hepaciviruses and pegiviruses and further enhance our understanding of the evolutionary history of hepatitis C virus and the human GB viruses (Quan et al. 2013).
In addition, PREDICT-Mexico sampled bats for dengue virus in landscapes that had undergone anthropogenic changes and also pristine areas in the Calakmul (Campeche) and Montes Azules (Chiapas) Biosphere Reserves in southern Mexico. Six bats (4.1%) tested positive for dengue serotype 2: four bats in Calakmul (two *Glossophaga soricina* bats, one *Artibeus jamaicensis* bat, and one *A. lituratus* bat) and two bats in Montes Azules (both *A. lituratus*; Sotomayor-Bonilla et al. 2014). No effect of anthropogenic disturbance on the presence of dengue virus in bats was detected in this study; however, the species of bats exhibiting positive results are abundant and well-adapted to disturbed habitats in the Neotropics (Sotomayor-Bonilla et al. 2014). The role of bats in the ecology of dengue virus is unknown.

**Investigation of Coronavirus Diversity in Bats**

More in-depth research was conducted to investigate coronavirus diversity in 42 different species of bats sampled at Campeche, Chiapas, and Mexico City. Including those samples screened in the initial testing using consensus PCR, a total of 1,046 samples from 606 individuals were used in this assessment.

Broadly reactive consensus PCR revealed coronavirus (CoV) sequences in 5.3% (32/606) of bats. Sequence analyses indicated high phylogenetic diversity and the presence of 13 distinct clades at the nucleotide level. Nine of the viruses clustered with known alphacoronaviruses, and four clustered with betacoronaviruses. One of the alphacoronaviruses (Mex_Cov-6) was closely related to a virus identified previously in an *Eptesicus fuscus* bat sampled on the Appalachian Trail in Maryland, USA (Donaldson et al. 2010). As a result of this research, the known geographical range of this virus was extended to southeastern Mexico. In addition, 12 novel CoVs were discovered in the bats. Seven novel CoV genotypes were discovered in 17 bats sampled in Campeche (17/240), six novel CoV genotypes were found in nine bats sampled in Chiapas (9/332), and two novel CoV genotypes were detected in six bats captured in Mexico City (6/34). Four of these novel coronaviruses were found in more than one bat.

**Clustering of Coronaviruses Based on Phylogenetic Relatedness of the Host Species.** Phylogenetic analyses demonstrated clustering of coronaviruses based on phylogenetic relatedness (i.e. taxonomic family) of the host species (Figure 3; Anthony et al. 2013). Alphacoronaviruses detected in phyllostomid bats cluster together, as do alphacoronaviruses discovered in miniopterid bats. Virus isolates from the closely related Vespertilionidae and Molossidae families of bats also cluster together. Similar patterns of clustering associated with host relatedness were observed in the betacoronavirus sequences. Viruses detected in rhinolophid bats cluster together, as do viruses found in the closely related Vespertilionidae and Molossidae families and Mormoopidae and Phyllostomidae families (Anthony et al. 2013).
Only one coronavirus sequence (Mex_CoV-5b) in this study has been detected in two distinct (but related) genera of bats (Anthony et al. 2013). Such findings have been reported previously, albeit rarely (Tong et al. 2009; Osborne et al. 2011; Lau et al. 2012), and demonstrate that CoVs can infect individuals from different genera/suborders. This sequence originated from a bat (Carollia sowelli) captured in a disturbed habitat. Additional viral discovery efforts are needed in this region to investigate whether disturbed habitats increase the risk or opportunity for viruses to spillover into new species, as previously suggested (Cottontailusen et al. 2009; Keesing et al. 2010; Suzán et al. 2012).

**Contributions to Targeted Surveillance of Bats in Saudi Arabia during MERS Outbreak.** PREDICT’s discovery of a betacoronavirus closely related (96.5% amino acid identity) to the MERS-CoV in a Nyctinomops laticaudatus bat captured for this study (Anthony et al. 2013; Figure 4) directed targeted surveillance of bats in Saudi Arabia. These efforts led to the discovery of a MERS-CoV sequence in a Saudi bat that was identical (100% nucleotide identity) to the virus from a human case-patient (van Boheemen et al. 2012). These findings highlight the importance of virus discovery and the need for risk characterization to enhance our understanding of the potential risk posed to animal and human health by these novel viruses.
Figure 4. Maximum-likelihood tree of a 329 bp fragment of the RdRp from all CoVs (red, a-CoVs; blue, b-CoVs; yellow, c-CoVs). Viruses discovered in this study are indicated by blue circles. The 2012 human b-CoV is indicated by an arrow, and clusters most closely to PMX-1247/Nyctinomops laticaudatus. Bar, 0.05 nucleotide substitutions per site. From Anthony et al. 2013.
REFERENCES


