excerpts from

Reducing Pandemic Risk, Promoting Global Health

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

The Bolivian portion of the Amazon represents 6.2% of the Amazon region and occupies half of the Bolivian territory. It is estimated that between 2000 and 2010, almost 765,000 hectares (1.6% of the Bolivian Amazon) were deforested for agriculture (RAISG 2012). Additionally, 15% of the Bolivian Amazon is under oil exploitation (23% located within indigenous territories), while 0.8% is being subjected to mining. Human migration from urban to forested areas is increasing, often following infrastructure development such as highway and dam construction, gas and oil operations, and timber extraction and mining, thereby bringing urban residents into close contact with wildlife.
The increasing disturbance of the Bolivian Amazon landscape is driving the emergence and re-emergence of zoonotic diseases. In the 1980s, 53% of 350 oil exploration workers were infected with cutaneous leishmaniasis during their incursions into the pristine forest in the Alto Beni region (Desjeux et al. 1987). In 2002, two cases of hantavirus pulmonary syndrome (HPS) were confirmed in two men living and working in an area of extensive anthropogenic disturbance where natural forest was being cleared to cultivate sugarcane (Carroll et al. 2005). More recently in 2013, three cases of HPS (two fatal) were diagnosed in workers from a new sugar mill in the Amazonian municipality of San Buenaventura; these were the first cases of hantavirus infections reported in La Paz (SEDES La Paz 2013).

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

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

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

Other local partners included:

- National Veterinary Service (SENASAG)
- General Directorate of Biodiversity and Protected Areas (DGBAP)
- Ministry of Public Health (MoH, Zoonoses, and Hantavirus/Hemorrhagic Fever Programs)
- Beni Regional Department of Health (SEDES Beni)
- Pan-American Health Organization (PAHO Bolivia)
- Instituto Nacional de Laboratorios en Salud (INLASA)
- Instituto de Investigación Técnico Científica de la Universidad Policial (IITCUP)
- Institute of Ecology from the Universidad Mayor de San Andrés, La Paz (IE - UMSA)
- Veterinary School of Universidad Católica Boliviana, Unidad Académica Campesina de Carmen Pampa (UCA-UAC CP)
- Biology School of Gabriel René Moreno University in Santa Cruz
- Museo de Historia Natural Noel Kempf Mercado
- La Senda Verde (LSV) wildlife rescue center
- Comunidad Inti Wara Yassi (CIWY) wildlife rescue centers
- Consejo Indígena del Pueblo Tacana (CIPTA)
- Consejo Regional T´simane Moseten (CRTM)
- Tierra Comunitaria de Origen Quechua-Tacana San José de Uchupiamonas
- Parque Nacional Madidi
- Reserva para la Biósfera Pilón Lajas
- Zoológico Municipal Vesty Pakos
- Red Boliviana Contra el Tráfico de Animales Silvestre (REBOCTAS)

**MAJOR ACHIEVEMENTS**

- Significantly improved capacity for disease outbreak response through a coordinated effort to identify and assist with the response to a yellow fever outbreak in free-ranging red howler monkeys in 2012 (see Success Stories for more information).

- Built laboratory capacity at the Institute of Molecular Biology (University of San Andres, IBMB lab) for viral genus/family level PCR on wildlife samples.
• Transferred diagnostic technology to the two most important national public health labs, Centro Nacional de Enfermedades Tropicales - National Center for Tropical Diseases (CENETROP) and Instituto Nacional de Laboratorios de Salud - National Institute for Public Health Laboratories (INLASA).

• Greatly increased knowledge and awareness of wildlife-origin zoonotic pathogens among public health agencies, organizations, and people at risk of exposure.

• Characterized the wildlife trade in Bolivia, in collaboration with the Bolivian Network against the Illegal Wildlife Trade (REBOCTAS), to provide enforcement agencies with information critically needed to identify priorities and strategies for trade regulation.

• Partnered with indigenous hunters and communities for wildlife disease surveillance of subsistence hunted animals, greatly expanding the reach of surveillance efforts and directly engaging a primary stakeholder group.

• Collaborated on the development of a National Zoonoses Surveillance Plan to formalize inter-agency cooperation for zoonotic disease surveillance, outbreak response, and risk communication.

SUCCESS STORIES

One Health Approach to Yellow Fever Outbreak

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

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

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

Development of a National Zoonoses Surveillance Plan

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

Intersectoral Cooperation

In order to develop a sustainable surveillance program, PREDICT facilitated inter-ministerial forums to design national strategies for wildlife disease surveillance. Key partnerships were formalized with nine institutions, including ministries (General Directorate of Biodiversity
and Protected Areas (DGBAP)), laboratories (IBMB, Instituto Nacional de Laboratorios en Salud (INLASA) and Instituto de Investigación Técnico Científica de la Universidad Policial - IITCUP), universities (Major University of San Andrés, La Paz (UMSA), Universidad Católica Boliviana, Unidad Académica Campesina de Carmen Pampa (UCA-UAC CP)), NGOs (WCS and BIOTA-PCMB), and civil organizations (La Senda Verde (LSV) wildlife rescue center).

**Training**

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

**Diagnostic Laboratory Capacity**

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

**SURVEILLANCE**

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

Figure 2. Number of animals sampled by taxa.
Table 1. Number of animals sampled according to targeted transmission interfaces.

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

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

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

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

**Local Media Surveillance**

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

**DISEASE OUTBREAK RESPONSE AND PREPAREDNESS**

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

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

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

The rabies outbreak in llamas in Potosi was believed locally to be associated with increasing attacks by foxes. The Bolivian government (MoH) and PAHO Bolivia invited SENASAG, the General Directorate of Biodiversity (DGB), and PREDICT to provide technical advice and expertise to help investigate and design a response strategy. Although local people were requesting fox culling, PREDICT promoted a preventive One Health approach: vaccination of both llamas and dogs and physical protection of llamas and livestock overnight with fencing. Culling of foxes
was discouraged to avoid ecological disruptions that could include, among other effects, massive increases in rodent populations and resulting damage to quinoa crops, the main livelihood of local people. Further studies carried out by PAHO and SENASAG showed that the rabies virus strain detected in the rabid fox was a domestic dog variant lending scientific evidence to PREDICT’s outbreak response strategy.

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

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

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

Additionally, key PREDICT local partners (PCMB, EHA, former WCS/PREDICT veterinarians, INLASA, and IE) joined efforts to promote a One Health vision among
university students, human and animal health workers, and biodiversity authorities. This vision is now being applied to modeling of sylvatic rabies distribution for early warning of potential bovine cases in Northern La Paz, as well as for evaluating hantavirus activity in rodents from the San Buenaventura area where the first cases of hantavirus in the La Paz department were reported in 2012 (SEDES La Paz, 2012).

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

Furthermore, information of viral findings in bat samples provided by PREDICT to the Bolivian health authorities has induced the MoH SEDES La Paz to increase survey efforts in patients with respiratory syndromes coming from the areas where bats tested positive for corona, hanta, and influenza viruses. Moreover, a new laboratory facility to detect tropical diseases (including zoonosis such as leptospirosis, hantavirus, and yellow fever) was recently established in the Amazon region of the La Paz department, where PREDICT focused its wildlife monitoring efforts. This local lab will significantly improve efforts to prevent diseases in areas where great biodiversity and anthropogenic changes converge.
Continued interest in pursuing activities initiated by PREDICT through other external funding, in addition to enhanced diagnostic capacity at CENETROP and INLASA, illustrate PREDICT’s positive impact in Bolivia through promoting science and local capacities to understand zoonotic disease ecology and a multidisciplinary team approach to the prevention of zoonotic disease outbreaks in the future.

REFERENCES


