excerpts from

Reducing Pandemic Risk, Promoting Global Health

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The Democratic Republic of Congo (DRC), located in the African Great Lakes Region of central Africa, is the second largest country in Africa with 2,345,408 km$^2$ of land and 65,710,000 inhabitants (World Bank 2012). The country includes the greater part of the Congo River Basin, which covers an area of almost 100 million hectares. Approximately 45% of the country is covered by primary forest, which is the second largest rain forest in the world after the Amazon. The flora and fauna in the DRC is the most diverse in the African continent (Lambertini 2000).

Numerous outbreaks of zoonotic infections have been reported in DRC, including the first known cases of Ebola virus and human monkeypox in 1976 and 1970, respectively. Since then, the majority of human monkeypox cases have been reported in DRC. Multiple outbreaks of Ebola virus disease have emerged, with up to 318 cases and 280 deaths reported in a single outbreak (WHO 1978). In addition, an outbreak of Marburg virus disease occurred at Watsa in northeastern DRC in 1998 with 154 cases and 128 deaths (Bausch et al. 2006). Additional zoonotic diseases are commonly and regularly reported, including yellow fever, plague, and dengue fever.

A significant percentage of diseases of public health importance in DRC are caused by pathogens originating from wildlife. Bushmeat hunting is common, and this activity has been associated with the emergence of Ebola viral disease. The index case of the first Ebola outbreak in DRC fell ill after consuming fresh and smoked antelope and nonhuman primate meat. In addition, the index case in the 2007 Ebola outbreak was suspected to have been infected from contact with bat

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meat: “the local population described a massive annual fruit bat migration; migrating bats settled in the outbreak area for several weeks and were massively hunted by villagers for consumption” (Leroy et al. 2009). The “putative first case bought freshly killed bats from hunters to eat” (Leroy et al. 2009).

In DRC, significant demographic and anthropogenic changes have occurred since the 1980s. These changes have likely increased contact between local populations and wildlife, especially in and around forested areas. Indeed, recurrent civil war and poverty have led to population displacements into the forest and consumption of bushmeat as a sole source of protein. In addition, forest clearing for farming, extractive industries, lodging, and firewood collection has favored increased contact with nonhuman primates, rodents, bats, and other wildlife species known to harbor zoonotic pathogens.

There was a critical need in DRC to establish a well-implemented surveillance system at the human-wildlife interface and to adopt a One Health approach to prevention and control of emerging and re-emerging infectious diseases. In response, PREDICT collaborated with the Government of DRC to create wildlife surveillance expertise and develop diagnostic laboratory and disease outbreak response capacities. Through these activities, PREDICT evaluated the diversity of wildlife viruses with potential for human infection and explored the implications of human and wildlife interactions on disease transmission and emergence in order to identify strategies for minimizing the risk of cross-species transmission.

In DRC, the PREDICT project built on the experience of human monkeypox and other active zoonotic surveillance programs that were initiated in concert with the Institut National De Recherche Biomedicale (INRB; English translation: National Institute of Biomedical Research) and the Kinshasa School of Public Health (KSPH) in 2000. These two institutions were the first in-country partners of PREDICT. The INRB, which is DRC’s national public health laboratory, provided space for a new laboratory to perform molecular tests on wildlife samples and a room for the project office, as well as scientific and administrative expertise. The director of INRB was the primary project point of contact at the DRC Ministry of Health. The KSPH facilitated administrative clearances with the DRC Government for the official recognition of the project and provided staff for the project. The Director of KSPH was the point of contact for the project at the Ministry of Higher Education and Scientific Research. PREDICT also collaborated with the Ministry of Health National Program against monkeypox and viral hemorrhagic fevers and the Central Direction for disease surveillance.

PARTNERS
PREDICT partners in DRC included Metabiota, Institut National de Recherche Biomedicale (INRB), Kinshasa School of Public Health, Ministry of Environment – Institut Congolais pour la Conservation de la Nature (ICCN), the Ministry of Health – Direction of Disease Surveillance, and USAID.

Other in-country partners included:

- Congolais pour la Conservation de la Nature (ICCN)
- Central Veterinary Laboratory of Kinshasa
- University of Lubumbashi
EPT partnerships included:

The PREDICT project conducted several activities in partnership with RESPOND, WHO, and the US CDC, including implementation of One Health activities by organizing meetings with key ministries; preparation of a curriculum for health, veterinary, and nurses training at high school and college levels; training of local staff in the Equateur province in investigation activities for human monkeypox; and responding to outbreaks in bonobos, chikungunya in Kinshasa, and during the 2012 and 2014 Ebola outbreaks.

WWF, through its “CARPE” and “PICBOU” projects, partnered with PREDICT by jointly visiting hotspot regions in the country, especially in the area of the Salonga National Park where the WWF and PREDICT staff worked together to sensitize local community members in the protection of nature and prevention of zoonotic infections by safe handling of bushmeat, avoiding risky behaviors, such as transporting and butchering found dead animals, and reporting abnormal die-offs among wildlife.

PREDICT regional collaborative laboratories:

The PREDICT project in DRC worked in partnership with the Centre International de Recherche Medicale de Franceville (CIRMF) in Gabon, the Center for Infection and Immunity (CII) at Columbia University in New York, USA, and the University of California, San Francisco.

PREDICT also collaborated with the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) monkeypox project in Kole.

A regional collaboration was established between PREDICT staff within the central African countries: DRC, Cameroon, Gabon, and Republic of Congo. This collaboration focused on training and sharing challenges and solutions, providing for “cross-pollination” and sustainability of One Health activities regionally.
MAJOR ACHIEVEMENTS:

• Assisted with the response to a variety of disease outbreaks in DRC, including Ebola virus disease. As a result of the success of PREDICT’s assistance during the outbreaks, the PREDICT laboratory at the National Institute of Biomedical Research was officially integrated into the viral hemorrhagic fever national surveillance system (see Success Stories for more information). PREDICT laboratory staff were included as members of the scientific and laboratory committee of the National Commission for Ebola Outbreak Response.

• The PREDICT laboratory diagnosed the first human samples from suspected cases of viral hemorrhagic fever (VHF) from Boende in the Equator province of DRC. The samples tested positive for Ebola Zaire using PREDICT protocols followed by sequencing. These results allowed the DRC Government to officially declare the 2014 Ebola Outbreak and to set up an outbreak response plan. The Ebola virus sequences were publically released via Genbank.

• Enabled discovery of a new, potentially highly-pathogenic human virus, the Bas-Congo virus (BASV) that caused an outbreak of viral hemorrhagic fever in the Mangala village and surrounding area in Bas-Congo Province (see Success Stories for more information).

• Engaged local communities by organizing regular village meetings during which community members were informed of surveillance activities, briefed on safe wildlife hunting and butchering techniques, and trained in the collection of samples from hunted wild animals (see Success Stories for more information).

• Developed infrastructure for and implemented a wildlife surveillance program. Equipment was procured and tested for utilization as a deployable laboratory that can be quickly set up in the field for outbreak response, including a real-time PCR machine, satellite phone, and a broadband satellite terminal to allow transmission of data from anywhere in the field – this equipment was deployed during the 2014 Ebola virus outbreak.

• Augmented diagnostic capacity through infrastructure improvements, including installation of a liquid nitrogen plant, which provided continuous cold-chain for sample collection throughout the country. PREDICT staff also optimized and implemented testing for 21 virus families.

• Sampled a total of 3,459 wild animals including bats, nonhuman primates, rodents and shrews, and animals from other taxa.
Assessed genomic diversity of the Monkeypox virus among people with primary and secondary cases of infection in DRC from 2005-2007. The research team detected four distinct lineages of the virus and discovered a deletion that resulted in gene loss that appears to correlate with human-to-human transmission and disease severity.

Detected a novel Simian Foamy Virus in healthy women from DRC. The Simian Foamy Virus (SFV) originated from the Angolan colobus (red-tailed monkey).

SUCCESS STORIES

Early detection of an Ebolavirus outbreak in the Democratic Republic of Congo

PREDICT assisted in the early detection of an outbreak of Ebolavirus in DRC in August, 2014. On August 11, a woman died in the village of Ikanamongo in Equateur Province of an unknown cause with symptoms of a hemorrhagic fever, and by August 18 reports of 24 suspected cases in nearby villages were received in the capital city of Kinshasa. A national response team went to the remote site and was able to deliver samples from eight individuals, who were in direct contact with the index case, to Kinshasa by August 22. However, key national virology staff were in West Africa at the time responding to governmental requests to assist in the Ebolavirus outbreak in that region. Consequently, the deputy director of INRB requested that the PREDICT laboratory, hosted at INRB, conduct the diagnostic testing. Ebolavirus was confirmed in samples from the outbreak using PREDICT protocols on August 23. Based on the early results of the PREDICT/INRB laboratory tests, the DRC government was able to enact disease control measures such as quarantine, dispatch of a mobile laboratory, and contract tracing, among other measures. Consequently, the prompt assistance of the PREDICT/INRB laboratory may have facilitated an early response and increased the likelihood of successful containment procedures.

Community Engagement

PREDICT staff worked closely with local community members to raise awareness on the risk of zoonotic infections, especially among hunting communities in forested areas. Community members participated in the surveillance activities by collecting bushmeat samples from their hunted wild animals. Participants received training on sample collection and were informed of the zoonotic pathogen risk associated with wildlife hunting and consumption. More than 2,000 bushmeat samples collected by community members were received at the laboratory in Kinshasa. The laboratory diagnostic results and implications to human health were shared at village meetings in order to increase local knowledge regarding zoonotic pathogens and the risk of spill-over to people through hunting, butchering, and consumption of bushmeat.
Integration of PREDICT into the Viral Hemorrhagic Fever National Surveillance System

PREDICT assisted with the response to a variety of disease outbreaks in DRC, including Ebola in 2012 and 2014, encephalomyocarditis virus in bonobos, *Brucella abortus* in cows from the Masisi District, and Chikungunya in Kinshasa. Due to the success and visibility of PREDICT’s assistance during these outbreaks, the PREDICT laboratory at the INRB was officially integrated into the national surveillance system for VHF. The laboratory received samples from VHF suspect cases for screening using PREDICT diagnostic protocols. Likewise, samples from people with fevers of unknown origin were provided to the PREDICT laboratory for pathogen detection and discovery.

Discovery of a Novel Human Pathogen Associated with Acute Hemorrhagic Fever in People

In collaboration with the Ministry of Health, the PREDICT DRC team, supported by the advanced diagnostic capability of the PREDICT Gabon team, obtained samples from survivors of a VHF outbreak in Mangala village, Bas-Congo Province. PREDICT used deep sequencing, a method for generating millions of DNA sequence reads from clinical samples, to discover a novel rhabdovirus (Bas-Congo virus, or BASV) associated with this outbreak of human cases of acute hemorrhagic fever in DRC.

Bas-Congo virus was detected in the blood of the lone survivor. The genome of BASV, assembled using a technique that was optimized through a larger PREDICT-funded viral discovery project at the University of California, San Francisco, revealed that the virus was very different from known rhabdoviruses (Grard et al. 2012). Antibodies to BASV were detected in one health care worker and the lone survivor. Although the source of the virus remains unclear, findings suggest that BASV is an emerging pathogen associated with acute hemorrhagic fever in Africa and may be spread by human-to-human contact. The discovery of BASV in Central Africa suggests that additional rhabdoviruses of clinical and public health importance await discovery, especially in these poorly investigated geographic regions. Active surveillance is needed to identify the source of infection and fully ascertain the public health significance of BASV infection.

CAPACITY BUILDING

Training

**Surveillance and Disease Outbreak Response.** PREDICT-DRC provided a variety of training sessions for project staff and collaborators to improve capacity for disease surveillance and outbreak response. PREDICT DRC staff received training on a broad range of topics, including but not limited to: wildlife capture and handling, specimen collection and processing, cold chain management, laboratory diagnostics and safety, biosafety and PPE use, and database management. Training on proper use of PPE was also provided to staff and participants from ministries and other collaborating organizations. Specific fit testing, using internationally standardized procedures, was conducted on individual staff members to ensure the appropriate PPE size and fit. In addition, two PREDICT staff and one scientist from the INRB were trained on biorisk management and shipping of Class 6.2 dangerous goods and dry ice, by the Sandia National Laboratories in the USA. These individuals are now equipped to train other in-country laboratory workers on these topics.
PREDICT also provided unique training opportunities to staff and officials from collaborating ministries, NGOs, and the national veterinary laboratory. Participants included both senior officials responsible for management and policy development and regional staff responsible for day-to-day management of wildlife conservation and veterinary services. Staff from the Ministry of Environment and Livestock were trained on wildlife capture and handling, wildlife sampling, specimen storage and transport, proper PPE use, and risk communication regarding suspected zoonotic disease cases and anomalous animal health events (i.e. disease outbreaks and unexpected mortality events). In addition, senior staff from the Ministry of Environment were trained on the use of the PREDICT assessment tool to assess new hotspot sites and new human-wildlife interfaces to be targeted for surveillance activities. Local veterinary technicians were trained on biosafety during animal disease outbreaks. Furthermore, the director of the National Program for Monkeypox and Viral Hemorrhagic Fevers received training on electrocardiography at the Kinshasa Provincial General Hospital, which provided a point-of-care diagnostic modality for assessing the impacts of human monkeypox and viral hemorrhagic fever infections on cardiac function of individuals during outbreaks.

**Train-the-trainer program.** To ensure sustainability of the enhanced surveillance capacity in DRC, PREDICT staff organized a train-the-trainer program: local staff in rural forested areas were trained as trainers of community members to expand the understanding of the origin of zoonotic diseases and measures to reduce the risk of infection and to provide opportunities to participate in disease surveillance efforts by providing bushmeat samples from hunted and butchered animals. Many villages in the Kasai-Oriental province participated in this program and provided bushmeat samples for testing. PREDICT staff organized regular village meetings during which community members were informed of surveillance activities, sensitized to biosafety measures that can be taken to reduce exposure to zoonotic pathogens during hunting and butchering activities, and trained in the collection of bushmeat samples from hunted wild animals. Several communities participated in the surveillance efforts by providing high quality samples from hunted nonhuman primates, rodents, and other wild animal species. In addition, communities were provided information on how to report suspected cases of monkeypox and VHF.
**Laboratory Improvements**

PREDICT provided important equipment to support surveillance activities, including PCR machines and other supplies to conduct laboratory analyses, and multiple freezers to improve storage capacity for biological samples. In addition, PREDICT installed a liquid nitrogen plant to provide continuous cold-chain for sample collection throughout the country. The laboratory was equipped to perform rapid serologic tests, DNA and RNA extractions, and conventional PCR assays.

PREDICT staff optimized and implemented testing of a full virus panel composed of 21 virus genera/families: arenaviruses, coronaviruses, filoviruses, flaviruses, enteroviruses, paramyxoviruses, rhabdoviruses, lyssaviruses, hantaviruses, alphaviruses, henipaviruses, bunyaviruses, retroviruses, influenza viruses, simian foamy viruses, herpes viruses, bocaviruses, poxviruses, adenoviruses, and polyomaviruses. The DRC laboratory staff also optimized a PCR amplification method for detection of specific viruses, including hepatitis B virus, Simian T-Cell Leukemia virus, encephalomyocarditis virus, and the newly discovered Bas-Congo virus.

Equipment was procured and tested for its utility as a deployable laboratory that can be rapidly applied for field use during an outbreak response. The equipment included a real-time PCR machine and technology to transmit data and communications from anywhere in the field, which allows for real-time reporting of results.

**SURVEILLANCE**

At the launch of the PREDICT project in DRC, the team conducted, in collaboration with DRC Government institutions, NGOs, and other partners, an assessment of current data and surveillance activities at the human-wildlife interface. This informed the selection of surveillance sites where high-risk wildlife, specifically bats, rodents, and nonhuman primates, have a high likelihood of contact with humans.

The PREDICT DRC field team coordinated with partners including government, NGOs, and communities to prioritize surveillance sites and conduct field efforts in eight out of the 11 provinces of the country. Samples were collected from hunted animals, nonhuman primates at sanctuaries (sick individuals and recently accessioned primates from the wildlife trade), rodents and bats in and around human dwellings and in areas with ecotourism, and wildlife surrounding disease outbreaks (Figure 1; Table 1). The PREDICT team sampled a total of 3,459 wild animals (Figure 2), and collected a total of 21 samples from humans with suspected VHF, two of which tested positive for Ebola Zaire virus.
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>1</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Ecotourism and recreational activities</td>
<td>24</td>
<td>18</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>16</td>
<td>49</td>
<td>397</td>
<td>3</td>
</tr>
<tr>
<td>Hunted wildlife</td>
<td>679</td>
<td>935</td>
<td>75</td>
<td>687</td>
</tr>
<tr>
<td>Wildlife trade</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Markets</td>
<td>46</td>
<td>1</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>217</td>
<td>7</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>Protected areas</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other high-risk interfaces</td>
<td>0</td>
<td>62</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1001</td>
<td>1072</td>
<td>684</td>
<td>702</td>
</tr>
</tbody>
</table>

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

The PREDICT staff provided expertise and assistance during five (three human and two animal) disease outbreaks

- **Ebola outbreak in Boende, 2014.** The PREDICT team was responsible for the initial diagnosis of Ebolavirus as the cause of the outbreak. The team confirmed that the outbreak in Boende was caused by the same species of Ebolavirus as the West Africa outbreak, but that it was not closely related, suggesting that the DRC outbreak was an independent spillover event. Based on the early results of the PREDICT laboratory analysis, the DRC government was able to enact disease control measures such as quarantine, dispatch of a mobile laboratory, and contract tracing, among other measures. Consequently, the early assistance of the PREDICT laboratory may have facilitated an early response and increased the likelihood of successful containment procedures. At the time of publication of this report, PREDICT continued to play a role in the outbreak response efforts (See Success Stories above).

- **Ebola outbreak in Isiro, 2012.** During the outbreak, the PREDICT team participated in the outbreak response with other national and international organizations. Samples were collected from nearby bats and rodents, as well as from nonhuman primates kept as pets. In addition, samples collected from pigs were also received for testing following rumors of a swine die-off. Local personnel from the Ministries of Environment and Livestock were trained in PREDICT field protocols, including wild animal sampling, sample shipment, personal protection, safe handling of animal carcasses, and reporting of animal health events. PREDICT staff was involved in regular government meetings held during the outbreak and provided expertise on implementation of a One Health approach to the response.

- **Brucella abortus outbreak in cows in the Masisi District, Eastern DRC.** In September-October 2010, a farmer with a herd of 1,000 cows experienced an outbreak of neonatal calf mortality. The PREDICT staff assisted the Central Veterinary Laboratory in Kinshasa with the response by coordinating sample shipment and testing. The Central Veterinary Laboratory isolated *Brucella abortus*. 
• **Chikungunya outbreak in humans and bonobos at the bonobo sanctuary in Kinshasa.** In January 2012, the PREDICT staff was called to the sanctuary Lola Ya Bonobo to investigate cases of illness in newly accessioned bonobos and sanctuary staff. After organizing a meeting with the director of INRB and Ministry of Health (MoH) staff, serological tests were performed on samples collected from the bonobos and staff. A diagnosis of chikungunya virus was made. The MoH and contingency were alerted of the results and control measures were implemented. The PREDICT team mobilized a team of entomologists who trapped mosquitos at the sanctuary for screening. Information was shared with the MoH official in charge of disease surveillance. All health facilities in Kinshasa were also made aware of the outbreak.

• **Encephalomyocarditis outbreak among bonobos at the bonobo sanctuary in Kinshasa.** From January through May 2012, four bonobos died at the sanctuary. PREDICT took the lead in coordinating the investigation and response to this outbreak. We arranged a veterinary visit to the site for a clinical investigation and shipped samples to CIRMF and the Center for Infection and Immunity (CII) at Columbia University, where they were screened for a wide range of pathogens. Specimens tested positive using molecular and histological assays for the encephalomyocarditis virus (EMCV). Control measures were implemented in and around the sanctuary to minimize the risk of infection.

• **Outbreak of viral hemorrhagic fever in Bas-Congo.** In May-June 2009, three suspect cases of VHF presented in the Bas-Congo province in western DRC. The first two cases died and the third one survived. All samples were negative for known viral hemorrhagic fever pathogens. PREDICT conducted an investigation in the area and collected samples from the lone survivor and his close contacts. These efforts led to the discovery of a novel virus, belonging to the rhabdovirus family, named Bas-Congo virus (BASV). PREDICT also collected wildlife samples in the immediate area of the outbreak to investigate potential reservoir species.

**VIRUS DETECTION AND CHARACTERIZATION**

PREDICT made it possible to discover a potentially highly-pathogenic human virus, the Bas-Congo virus (BASV), a novel rhabdovirus, that caused an outbreak of viral hemorrhagic fever in the Mangala village and surrounding area, Bas-Congo Province, via deep sequencing in collaboration with the University of California, San Francisco (Grard et al. 2012).

In addition, PREDICT researchers assessed the genomic diversity of the Monkeypox virus in samples obtained from humans with primary and secondary cases of infection from 2005-2007 (Kugelman et al. 2014). The results revealed four distinct lineages and a deletion that resulted in gene loss that appears to correlate with human-to-human transmission and disease severity. The results suggest frequent spillover of the viruses from animals to people and potential increased adaptation to humans (Kugelman et al. 2014).

Further, PREDICT researchers screened a large population of rural 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 commonly hunted nonhuman primate species. Unlike previous studies, women were not at lower risk of infection with simian foamy viruses relative to men (Switzer et al. 2012). Men commonly hunt while women are responsible for food preparation, suggesting that exposure may be as or more frequent with bushmeat preparation compared to hunting.

REFERENCES


