excerpted from
Reducing Pandemic Risk, Promoting Global Health

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The Republic of Congo (RoC) is home to a number of key wildlife species such as chimpanzees, gorillas, and forest elephants. Important conservation zones protect large tracts of forest, which make up 66% of the area of the 341,500 km$^2$ of the country (World Bank 2014) and are especially dominant in the north. These remote forests provide habitat for the highest known densities of lowland gorillas, as well as other endangered high profile species. RoC encompasses a diversity of landscapes, including the basin of the Congo River.

In RoC, 36% of the population of 4.3 million people lives in rural areas and 31% of the land area is agricultural (World Bank 2014). Rural communities rely heavily upon hunting for food and for income. Bushmeat is consumed by subsistence hunters and also sold in markets. Hunting can expose individuals to infections from wildlife through bites and scratches, direct contact with animal blood and bodily fluid, and ingestion of un/under-cooked meat. Various wildlife pathogens, including new retroviruses, have been detected in people who hunt and butcher wild animals in Central Africa (Wolfe et al 2004, 2005; Zheng et al 2010). Understanding the diversity of pathogens in wildlife in RoC aids in preventing or limiting the emergence of new infectious diseases and, thus, reduces the threat of pandemics.

PREDICT collaborated with the Government of RoC to build wildlife surveillance capacity. This partnership allowed PREDICT to evaluate the diversity of wildlife viruses with the potential for human infection, as well as to explore the implications of human and wildlife interactions on disease transmission and emergence. A better awareness of potential zoonotic viruses allows public health authorities and communities to consider measures to reduce risk of infection and to help with the rapid identification of viruses that may cross over from wildlife to humans.
PARTNERS
PREDICT partners in RoC included:

• African Parks
• Global Viral (GV)
• Global Viral Cameroon (GV-C)
• IUCN Elephant Bushmeat Pilot Project
• Laboratoire Nationale de Sante Publique/National Public Health Laboratory (LNSP/NPHL)
• Laboratoire de Diagnostique Vétérinaire de Brazzaville/Brazzaville Veterinary Diagnostic Laboratory (LDVB)
• Metabiota
• Ministère de l’Agriculture/Ministry of Agriculture
• Ministère de l’Economie Forestière et du Développement Durable/Ministry of Forestry Economics and Sustainable Development
• Ministère de la Défense Nationale/Ministry of National Defence
• Ministère de la Santé et de la Population/Ministry of Health and Population
• Ministre de la Recherche Scientifique et de l’Innovation Technologique/Ministry of Scientific Research and Technological Innovation
• National Institute of Allergic an Infectious Diseases, National Institute of Health, Rocky Mountain Labs (NIAID/NIH-RML)
• National Institute of Allergic and Infectious Diseases, National Institutes of Health, Vaccine Research Center (NIAID-VRC)
• Projet Nouabale-Ndoki (PNN)
• Université Marien NGOUABI/Marien NGOUABI University (UMN)
• Wildlife Conservation Society (WCS)
• World Health Organization Regional Office for Africa (WHO AFRO)
• USAID

MAJOR ACHIEVEMENTS
• Established a community wildlife surveillance system in areas of high risk for Ebola virus emergence by educating hunters on the best procedures for reporting animal carcasses found in the forest (see Success Stories for more information).
• Published evidence-based animal surveillance recommendations for a series of human Ebola virus outbreak investigations in Africa. Guidance was provided on susceptible target species and prioritization of assays to rapidly detect the presence of Ebola virus in animal reservoirs (see Success Stories for more information).
• Tested fruit bats from a bushmeat market for paramyxoviruses to investigate risk to humans associated with hunting and preparation and consumption of bats. Four samples yielded Henipavirus-related sequences that formed at least 3 distinct groups in the Paramyxoviridae family.

• Conducted research to optimize noninvasive sampling strategies for disease surveillance in gorilla populations in Central Africa (see Success Stories for more information).

• Enhanced disease surveillance capacity and coordination among animal and public health officials to ensure communication across health sectors. PREDICT coordinated the translation of classroom-based training into practical experience through facilitation of field surveillance activities led by teams from the Veterinary Diagnostic Laboratory Brazzaville (LDVB) and the National Public Health Laboratory (LNSP) and mentored by technicians experienced in wildlife surveillance and PREDICT protocols.

SUCCESS STORIES

Dead or Alive: Animal Sampling During Ebola Hemorrhagic Fever Outbreaks in Humans
PREDICT provided evidence-based animal surveillance recommendations for a series of human Ebola hemorrhagic fever outbreak investigations in Africa (Olson et al. 2012a), including information on susceptible target species, guidance on animal sampling for disease outbreaks occurring in resource-limited regions, and diagnostic assays to be prioritized to rapidly assess the presence of Ebola virus in animal reservoirs. Specific recommendations for targeted surveillance aimed to identify potential sources of transmission from animals to humans included: 1) investigation of all wildlife morbidity and mortality events; 2) sampling of animal carcasses (vs. live animals) for a higher likelihood of Ebola virus recovery; 3) surveillance of certain bat species using large sample sizes (n > 100); and 4) prioritization of dogs and pigs among domestic animals for screening for virus and antibodies (i.e. previous exposure; Olson et al. 2012a).

Building Hunter Networks to Help Detect Ebola Outbreaks in the Republic of Congo
The northern boundary of Odzala Kokoua National Park in the Republic of Congo is at high risk for Ebola emergence. PREDICT established a community wildlife surveillance network in the area by educating hunters on the best procedures for reporting animal carcasses found in the forest. The goal of this community-based surveillance system is to allow for continued, sustainable monitoring of potential outbreaks.

Hunters represent about 22% of the population in this highly susceptible region. The team visited 512 hunters in 27 villages that had previously experienced Ebola epidemics, reinforcing the importance of reporting wildlife morbidity or mortality events. During the project, hunter networks reported numerous gorilla carcasses prompting PREDICT teams to be mobilized for sampling.

Optimization of Noninvasive Methods for Wildlife Disease Surveillance in Nonhuman Primates
PREDICT scientists also conducted research to optimize noninvasive sampling techniques for conducting disease surveillance for wildlife taxa that are difficult to locate and capture (i.e. nonhuman primates). Simulation modeling was used 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 (Olson et al. 2012b). A mixed sampling design, combining traditional transect and directed reconnaissance designs, 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).

CAPACITY BUILDING

Training

PREDICT presented unique training opportunities for staff, collaborating ministries, the national public health laboratory, and the national veterinary diagnostic laboratory (Laboratoire de Diagnostique Vétérinaire de Brazzaville - LDVB). Training was provided to eight technicians from the National Veterinary Diagnostic Laboratory and staff from regional livestock and wildlife authorities on the use of PPE, wildlife capture and handling, and sample collection and storage. This training was held prior to field surveillance activities led by the LDVB and the National Public Health Laboratory and was supported by PREDICT Cameroon staff. PREDICT mentored LDVB staff during the planning and execution of wildlife sampling. Brazzaville Veterinary Diagnostic Laboratory staff led the planning and execution of field surveillance activities, and local and central ministerial staff observed elements of the surveillance in action. Training conducted in the classroom was augmented through field-based sessions, which reinforced the didactic material and provided practical experience.

PREDICT also provided laboratory training to technicians based at the National Public Health Lab and from the Université Marien Ngouabi in Brazzaville. The trainings covered topics including surveillance techniques, wildlife capture and sampling, sample handling and transport, laboratory analyses and management, laboratory best practices, and database management.

Photo left: PREDICT team undertaking a training of staff from Brazzaville Veterinary Diagnostic Laboratory and National Public Health Laboratory on PREDICT sample collection protocols in Ouesso, RoC.

Photo right: Personal protective equipment demonstration by PREDICT in Ouesso, RoC for staff from Brazzaville Veterinary Diagnostic Laboratory and National Public Health Laboratory.
Training was conducted at the National Public Health Laboratory in Brazzaville by project staff and collaborators from Tulane University, National Microbiology Laboratory, PHAC SPP, and Laboratory of Virology, NIAID/NIH RML. Additional training was conducted at the PREDICT laboratory in Yaoundé Cameroon. PREDICT demonstrated in-country capacity for PCR and its field portability to representatives of RoC Ministries of Health, Ministry of Forest Economy, Ministry of the Environment, Ministry of Defense, and Ministry of Agriculture.

Staff from PREDICT RoC also participated in training at the Pan-African Sanctuary Alliance (PASA) annual meeting in South Africa covering topics such as necropsy, new laboratory analytical techniques, introduction to disease risk analysis for primate reintroduction programs, disease contingency planning, and scientific writing and presentation skills.

**Laboratory Infrastructure and Diagnostics**

At the National Public Health Laboratory, PREDICT made investments in laboratory infrastructure with support from the NIH. PREDICT developed capacity for laboratory diagnostics, including detection of various strains of Ebola and Marburg viruses (i.e. Zaire ebolavirus, Sudan ebolavirus, Côte d’Ivoire ebolavirus, Bundibugyo ebolavirus, and Marburg virus). This diagnostic panel was expanded to include yellow fever virus, poliovirus, dengue virus, Rift Valley fever virus, malaria, *Mycobacterium ulcerans*, measles virus, and monkeypox virus. Specimens were also tested at various partner laboratories including CIRMF, Columbia University Center for Infection and Immunity (CII), University of California, Davis, and the INRB PREDICT laboratory in Kinshasa.

**SURVEILLANCE**

A range of high-risk human-wildlife interfaces were investigated in RoC (Figures 1 and 2). Sites and species targeted for wildlife surveillance were chosen based on risk of disease outbreaks from known pathogens, such as Ebola virus, and novel pathogens that could be transmitted from wildlife to people through hunting and butchering and contact in or around human dwellings in rural areas (Table 1). An additional area of focus included extractive industry sites. PREDICT coordinated with rapid bio-assessment missions and other US government agencies (NIAID/NIH Rocky Mountain Labs and PHAC Special Pathogens Program) conducting research in areas of priority for surveillance, in particular sites in areas impacted by extractive industries.
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>Ecotourism and recreational activities</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Extractive industries</td>
<td>0</td>
<td>59</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>3</td>
<td>69</td>
<td>93</td>
<td>1</td>
</tr>
<tr>
<td>Hunted wildlife</td>
<td>91</td>
<td>34</td>
<td>54</td>
<td>248</td>
</tr>
<tr>
<td>Markets</td>
<td>88</td>
<td>20</td>
<td>43</td>
<td>115</td>
</tr>
<tr>
<td>Wildlife being studied</td>
<td>0</td>
<td>31</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pristine habitat</td>
<td>168</td>
<td>101</td>
<td>265</td>
<td>2</td>
</tr>
<tr>
<td>Other high-risk interfaces</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>352</strong></td>
<td><strong>317</strong></td>
<td><strong>539</strong></td>
<td><strong>367</strong></td>
</tr>
</tbody>
</table>

PREDICT-RoC also investigated the best noninvasive sampling methods for free ranging primates, including optimal strategies for fecal sample collection from gorillas in the dense forests of Central Africa (see Success Stories for more information; Olson et al. 2013).

**Community Hunter Wildlife Surveillance Network**

A group of 285 hunters among ten villages aided the team in identifying bat roosting sites, including a number of roosts in caves and mines. Samples were also collected in villages and in markets from primate, swine, carnivore, and rodent carcasses destined for human consumption. This opportunistic collection included some species outside the priority wildlife taxa for PREDICT, such as duikers and hogs, because these species have been implicated in Ebola virus ecology.

PREDICT also established a community-based animal morbidity and mortality surveillance system in areas of high risk of Ebola emergence. A total of 512 hunters in 27 villages were visited in regions previously experiencing Ebola epidemics and were provided training on how to report wildlife morbidity or mortality events. Hunter networks reported numerous gorilla carcasses during the course of PREDICT, and teams were mobilized for sampling.

**DISEASE OUTBREAK RESPONSE AND PREPAREDNESS**

PREDICT-RoC reported key information on a number of suspected hemorrhagic disease outbreaks in humans and wildlife to the RoC Ministry of Health. During a suspected hemorrhagic fever disease event in Mokouangonda, PREDICT and WHO AFRO supported the MoH in their response by mobilizing a response team. This team conducted interviews and collected patient history, clinical data, epidemiologic information, and biological samples. Additionally, PREDICT mobilized four field teams in the areas surrounding the outbreak to collect samples from wildlife and conduct reconnaissance surveys to evaluate disease outbreak effects on large primates and other wildlife. PREDICT facilitated communication with other field partners, such as WCS and African Parks throughout the course of the project.
PREDICT also assisted with the response to an outbreak in Likuala, Northern Congo by facilitating the transport and testing of clinical samples at the Centre International de Recherche Médicales de Franceville (CIRMF). The samples were screened for Chikungunya, dengue, yellow fever, Rift Valley fever, West Nile, Zika, and O’nyong nyong viruses.

In addition, PREDICT participated in a workshop with government officials (MoH, Epidemiology and Hygiene Service, and Avian Influenza project), WHO, and local communities with the aim of improving reporting speed, transparency, and response during disease outbreaks.

**VIRUS DETECTION AND CHARACTERIZATION**

**Henipavirus-related Sequences in Fruit Bats from a Bushmeat Market in the Republic of Congo**

Bats are hosts for the zoonotic paramyxoviruses Hendra virus and Nipah virus, which have caused severe disease outbreaks among humans and livestock. PREDICT investigated the risk of cross-species transmission of paramyxovirus associated with hunting, preparation, and consumption of bats in the Republic of Congo. Samples obtained from live straw-colored fruit bats (*Eidolon helvum*) captured by hunters for bushmeat were screened for paramyxoviruses (i.e. respirovirus, morbillivirus, and henipavirus) 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 these bat paramyxovirus sequences. Phylogenetic analysis revealed no spatial distinction between the sequences in the bats 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). While there are no documented cases of human infection associated with bat paramyxoviruses in Africa, antibodies to henipavirus or henipa-like viruses have been detected in domestic pigs, which are amplifying hosts for people and may indicate exposure to virus in bat excreta (Hayman et al. 2011). Additional research on virus-host ecology and assessment of at-risk persons are needed to evaluate the zoonotic disease risk of these viruses.

**REFERENCES**


