excerpts from

Reducing Pandemic Risk, Promoting Global Health

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The Gabonese Republic is located on the Gulf of Guinea in Central Africa, crossed by the equator and bordered by the Republic of Congo, Cameroon, and Equatorial Guinea. This country occupies 270,000 km$^2$ of land, three-fourths of which is covered by tropical forest. Thirteen national parks have been created in Gabon, representing 10% of the territory, which includes the Lopé National Park, a UNESCO World Heritage site. In the western and southeastern regions of the country, coastal lowlands and savannah landscapes are the predominant ecosystems, respectively. Watersheds of the Ogooué River occupy a significant part of the country (Encyclopedia Britannica 2014). The flora and fauna in Gabon spans a remarkable biodiversity, undoubtedly one of the most diverse in the world. About 200 different mammalian species are found in Gabon, including rare species such as pangolins and sun-tailed monkeys. In addition, the country’s forests are an important refuge for gorillas and chimpanzees.

According to the United Nations, Gabon is a prosperous country with the highest human development index in the African continent. Although intensive agriculture and ranching are poorly developed, there are significant forest resources. However, significant anthropogenic disturbance has occurred in the forests due to exploitation of oil, minerals, and hydropower. Despite the official figures, a significant proportion of the population remains poor in Gabon. The population is estimated to be over 1,500,000, consisting of more than 40 ethnic groups. The country has a very low population density (six inhabitants per km$^2$, as compared to 37 per km$^2$ for the African continent as a whole; Encyclopedia Britannica 2014). However, half of the population lives in the two major cities: Libreville, the capital in north-west and Port-Gentil, the economic capital and main harbor in the east, positioning Gabon as one of the most urbanized countries in Africa.
In 1979, the Centre International de Recherches Médicales de Franceville (CIRMF) was created in Gabon and has served as a modern research facility for Central Africa. CIRMF focused its activities in the early 1980s on HIV/AIDS and malaria; the two major pandemics affecting Africa. In the late 1990s, emerging infectious disease threats, such as Ebola virus disease, Chikungunya fever, and influenza A (H1N1) led CIRMF to adopt the One Health concept. Moving forward, CIRMF conducted scientific research programs that focused on infectious human pathogens and their zoonotic origins.

Hunting of wildlife is an important cultural and economic activity in Gabon. Bushmeat is a major source of dietary protein and income for rural communities. However, hunting carries substantial risk for inter-species transmission of zoonotic pathogens and is assumed to be the mechanism by which HIV and other dangerous zoonotic pathogens first spilled over into humans (Pike et al. 2010). Several pathogens have emerged in the forests of Gabon, including viruses responsible for hemorrhagic fever outbreaks. Human and animal outbreaks of Ebola have occurred in recent decades, and Marburg virus has been isolated from fruit bats in Gabon.

PREDICT partnered with CIMRF to enhance the country’s wildlife surveillance, diagnostic laboratory, and outbreak response capacities. PREDICT-Gabon focused on detection of zoonotic pathogens of wildlife origin in areas where anthropogenic change and human behaviors have resulted in increased contact between wildlife and human populations, and therefore a heightened risk of inter-species transmission.

**PARTNERS**

PREDICT partners in Gabon included:

- Centre International de Recherches Médicales de Franceville (CIRMF)
- Metabiota
- Agence Nationale des Parcs Nationaux (ANPN)
- Conseil Africain et Malgache pour l’Enseignement Supérieur (CAMES)
- Centre National de la Recherche Scientifique (CENAREST)
- Ecole Doctorale Régionale d’Afrique Centrale (EDR)
- Food and Agriculture Organization (FAO)
- Institut National Supérieur d’Agronomie et de Biotechnologie (INSAB)
- Institut Pasteur (IP)
- Institut pour la Recherche et le Développement (IRD)
- Laboratoire National de Santé Publique (LNSP)
• French Ministry of Foreign Affairs (MAE)
• USAID
• Gabonese Ministry of Education and Research (MoER)
• Gabonese Ministry of Health (MoH)
• Gabonese Ministry of Water and Forest (MoWF)
• Projet Grands Singes (PGS)
• Université des Sciences de la Santé - Omar Bongo (USS-OB)
• Université des Sciences et Techniques de Masuku (USTM)
• Wildlife Conservation Society (WCS)
• Word Health Organization (WHO)

MAJOR ACHIEVEMENTS
• Served as a regional diagnostic laboratory for virus detection and characterization and made significant advancements in our understanding of highly pathogenic viruses emerging in Central Africa (see Success Stories for more information).
• Characterized the Zaire ebolaviruses from the 2007 and 2008 outbreaks in Luebo, Democratic Republic of Congo (DRC; see Success Stories for more information).
• Confirmed results produced by the Institut National de Recherche Biomedical (INRB) in DRC that the 2014 Ebola outbreak was distinct from the West Africa Ebola virus outbreak, and completed full genome sequencing of the virus.

The PREDICT field team collects specimens from bats captured at the Belinga caves near Makokou.
• Enhanced disease surveillance capacity and implemented a surveillance network that incorporates both the animal and human health sectors, collecting specimens from 3,491 animals.

• Established a national surveillance network for specific syndromes including hemorrhagic, febrile, enteric, and flu-like illnesses in association with the high Gabonese authorities within the nine Gabonese provinces; a reference physician was identified in each regional hospital to facilitate sample collection.

• Developed an animal disease reporting system that meets international standards in partnership with the Centre National de la Recherche Scientifique, the Ministry of Health, and the Agence Nationale des Parcs Nationaux.

• Expanded implementation of advanced molecular laboratory diagnostics to detect and characterize both known and novel zoonotic viruses in wildlife and optimized and implemented 21 viral family testing protocols.

SUCCESS STORY

Regional Diagnostic Laboratory for Human and Wildlife Disease Events

As of 2014, the PREDICT in-country partner laboratory, CIRMF, had increasingly become the reference diagnostic testing facility for Central Africa. In particular, specimens from unusual and/or highly pathogenic human and wildlife disease cases were routinely forwarded to CIRMF for assistance in detection and characterization of the etiologic agent.

During PREDICT, CIRMF collaborated on a number of investigations during suspected VHF outbreaks within the Central African region. For example, PREDICT and its partners reconstructed the genome of a novel rhabdovirus (Bas-Congo virus, or BASV) associated with three human cases of acute hemorrhagic fever in DRC in 2009 (Grard et al. 2012). The rhabdovirus family had never been documented to cause hemorrhagic fever in people. Given the severity of the associated syndrome, this virus is potentially a new BSL-4 virus, representing a credible threat to public health. Identification of this virus expands the panel of viruses in diagnostic tests when viral hemorrhagic fever is suspected, improving our ability to identify etiologic agents in similar outbreaks.

In addition, PREDICT characterized the Zaire ebolaviruses from the 2007 and 2008 outbreaks in Luebo, DRC (Grard et al. 2011a). The two Luebo ebolaviruses were found to be distinct from previous ebolaviruses characterized in DRC and in the Gabon-Republic of the Congo area, suggesting that many Ebola virus outbreaks do not result from viral spread from previously identified foci. Instead, the outbreaks likely arise from independent emergence events, and local wildlife populations (most likely bats) become infected and allow viral persistence and reemergence from year to year (Grard et al. 2011).

Furthermore, CIRMF used phylogenetic analysis to investigate an isolated human case of Crimean-Congo hemorrhagic fever (CCHF) in DRC in 2008. The analysis suggested that CCHF virus circulation in Central Africa has been occurring for a long time, despite the absence of reported human cases, and that many human infections have likely been overlooked. The sporadically reported human cases are associated with a sylvatic cycle in Central Africa where deforestation may heighten the risk of re-emergence (Grard et al. 2011b).
CAPACITY BUILDING

Laboratory Diagnostic Capacity

During the PREDICT project, CIRMF diagnostic infrastructure was substantially enhanced through installation of new laboratory equipment that allowed for rapid automation and high-throughput techniques for pathogen discovery and characterization. PREDICT-Gabon optimized and implemented protocols to detect both known and novel viruses in 22 viral families/genera of high-consequence (alphaviruses, arenaviruses, astroviruses, bunyaviruses, coronaviruses, enteroviruses, hantaviruses, henipaviruses, hepadviruses, flaviviruses, lyssaviruses, orbiviruses, paramyxoviruses, phleboviruses, rhabdoviruses, influenza viruses, retroviruses, adenoviruses, bocaviruses, herpes viruses, poxviruses, and polyomaviruses). These protocols were used to screen clinical specimens from both wildlife and humans. Testing was facilitated through development of universal control materials allowing test validation without cultivation or shipment of viruses, or maintenance of unstable RNA virus control material.

Training

A series of trainings were provided for personnel, including wildlife capture and sampling, bushmeat sampling, specimen handling and shipment, PPE use and biosafety, laboratory methodology, laboratory safety, and data management. One member of the PREDICT Gabon team received regional training from the Cameroon team in order to optimize the PREDICT protocols in Gabon, refine wildlife collection techniques, share laboratory experiences, and build a regionally sustainable collaboration among PREDICT teams. This training was applied and was conducted within the laboratory and during field sampling trips with the Cameroon team. The PPE training was extended to participants from ministries and other collaborating organizations. As part of this training, specific fit testing using internationally standardized procedures was conducted on individual staff members to ensure the appropriate PPE fit. PREDICT laboratory staff was comprehensively trained in all steps of sample processing and pathogen discovery techniques. Molecular biology expertise was improved locally and used for the duration of the project. In addition, PREDICT staff contributed to the training of graduate students, healthcare workers, and local scientists on a range of topics, including specimen collection, transport, processing, and laboratory analysis.

SURVEILLANCE

PREDICT enhanced surveillance capacity in Gabon by augmenting resources to conduct active and opportunistic sampling of high priority wildlife taxa. Samples were collected throughout the country from bats, rodents, nonhuman primates, and ungulates at high-risk disease transmission interfaces between wildlife and humans (Figure 1 and Table 1). In addition, bushmeat samples were collected from hunters and markets. During the PREDICT project, samples were collected from 3,491 animals (Figure 2; bats, rodents, nonhuman primates, and ungulates). A strategic sample selection system was utilized to prioritize testing based on taxa, specimen type, and risk interface targeted to maximize discovery of viruses of public health significance.
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>0</td>
<td>0</td>
<td>0</td>
<td>340</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>0</td>
<td>322</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>Hunted wildlife</td>
<td>20</td>
<td>21</td>
<td>200</td>
<td>45</td>
</tr>
<tr>
<td>Pristine habitat</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Protected areas</td>
<td>0</td>
<td>140</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>Other high-risk interfaces</td>
<td>3</td>
<td>158</td>
<td>2102</td>
<td>19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>23</strong></td>
<td><strong>641</strong></td>
<td><strong>2393</strong></td>
<td><strong>434</strong></td>
</tr>
</tbody>
</table>

In addition to wildlife disease surveillance, a national syndromic surveillance system was established in association with the high Gabonese authorities within the nine Gabonese provinces; a reference physician was identified in each regional hospital to facilitate sample collection and shipment to CIRMF. Hemorrhagic, febrile, enteric, and flu-like syndromes were targeted.

Furthermore, PREDICT-Gabon contributed to an animal disease reporting system for national authorities responsible for animal disease management, including the Centre National de la Recherche Scientifique, the Ministry of Health, and the Agence Nationale des Parcs Nationaux. This was the first coordinated mechanism for animal disease reporting in the country.

PREDICT expanded the implementation of advanced molecular laboratory diagnostics to detect and characterize known and novel viruses. CIRMF developed national partnerships with ministries, universities, and other public health facilities throughout the country.

**DISEASE OUTBREAK RESPONSE AND PREPAREDNESS**

PREDICT and its partner CIRMF assisted in the diagnosis of unusual human and wildlife diseases regionally. Outbreak response was coordinated in partnership with the Gabonese ministries. Multidisciplinary field teams from CIRMF collected specimens during wildlife and/or human disease outbreaks and deployed a mobile laboratory with biosafety equipment during suspected outbreaks within the region. Furthermore, CIRMF benefited from a BSL-4 rated high containment laboratory and now has the capacity to employ next generation sequencing technology during outbreak investigations.

From 2009 to 2012, wildlife and human samples collected during acute hemorrhagic fever outbreaks in the DRC were routed to CIRMF for analysis. Samples were tested for Zaire ebolavirus, Sudan ebolavirus, Ivory Coast ebolavirus, Bundibugyo ebolavirus, Marburg virus, Lassa virus, CCHF virus, Rift Valley fever virus, yellow fever virus, dengue virus, and Chikungunya virus. PREDICT protocols were also used to test for novel viruses. In 2010, a novel rhabdovirus (Bas-Congo virus, or BASV) was discovered during a VHF outbreak (Grard et al. 2012), suggesting the emergence of a new pathogen associated with acute hemorrhagic fever in Africa. Bas-Congo virus was added to the panel of viruses that samples were screened for in hemorrhagic fever cases.
In 2012, samples collected from several Bonobos in DRC, which had died from cardiopulmonary disease and hemorrhage, were sent to CIRMF for analysis. These specimens were screened for a broad range of pathogens and were found to be negative, except for one sample, which tested positive for adenovirus. The bacteriological analyses revealed that the Bonobos’ deaths were likely the result of bacterial sepsis.

REFERENCES


