excerpts from
Reducing Pandemic Risk, Promoting Global Health

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Uganda is a highly populous nation in east-central Africa with a long-standing and well-established network of national parks. These parks provide protection for thriving populations of wildlife, all of which are surrounded by intensive animal agriculture and dense human communities, resulting in significant wildlife-livestock-human interactions. There are very few “buffer zones” between parks and land cultivated for crops or animals, which means that biodiversity is highly influenced by human use of landscapes. For example, Uganda is home to nearly half the world’s population of mountain gorillas, for which there is a thriving ecotourism industry that brings thousands of local and foreign people into contact with human-habituated gorillas (Gorilla Doctors 2014). Wildlife, including mountain gorillas, exit protected areas to forage in nearby cultivated fields, thereby coming into direct and indirect contact with people and domestic animals at the park margins. Additionally, a burgeoning cave tourism industry in Uganda brings local people and tourists into close contact with multiple species of bats.

Uganda has experienced several high-profile outbreaks of emerging infectious diseases (EIDs) in recent years, and intensive surveillance and research on the ecology of some of the more high-profile EIDs, such as those caused by filoviruses (e.g. Ebola virus, Marburg, and Bundibugyo; Polonsky et al. 2014), have rightfully focused the world’s attention on Uganda as a global “hotspot” for disease emergence. These factors, coupled with the infrastructure and efforts by multiple institutions and organizations (e.g. Uganda Wildlife Authority, Makerere University College of Veterinary Medicine, Gorilla Doctors) in disease monitoring and surveillance have facilitated the development and implementation of a comprehensive wildlife zoonoses surveillance program by PREDICT in Uganda.
Uganda’s strong foundation of academic and public health networks with partnerships throughout the region have great potential for successful application of a One Health approach to emerging pandemic threats in Uganda and throughout the East Congo Basin. The PREDICT project in Uganda further improved capacity to conduct wildlife disease surveillance in order to better characterize zoonotic disease risks and to support the Ugandan government in its disease outbreak response and preparedness efforts.

PARTNERS
PREDICT-Uganda was implemented by Gorilla Doctors, a partnership between the non-governmental organization Mountain Gorilla Veterinary Project (MGVP, Inc.) and the University of California, Davis. Key collaborating partners included Makerere University College of Veterinary Medicine, USAID, Animal Resources and Bio-security (COVAB) and the Uganda Wildlife Authority (UWA).

Makerere University’s COVAB is Uganda’s premiere academic institution of veterinary science and teaching and service delivery and is a semi-autonomous Institution of Higher Learning supported by the Government of Uganda. The mission of COVAB is to provide innovative teaching, learning, research, and services responsive to national and global needs. MGVP, Inc. is co-located within the COVAB complex. COVAB houses the Makerere University Walter Reed Project’s fully-equipped and staffed influenza research laboratory that conducts routine avian influenza surveillance and diagnostics in Uganda, and with which MGVP, Inc. collaborated on laboratory diagnostics support and services for PREDICT.

The Uganda Wildlife Authority (UWA), created in 1996, is the official government body charged with management of the wildlife resources of Uganda. UWA’s Veterinary Unit has the formidable charge of health management for all wildlife, including the mountain gorillas. As a flagship species, and the cornerstone of Uganda’s ecotourism industry, the endangered mountain gorilla features prominently in the activities of UWA and its Veterinary Unit. UWA recognizes that collaboration and cooperation are needed to effectively manage and protect wildlife.

Other important partners in implementation of PREDICT activities in Uganda include: The Ngamba Island Chimpanzee Sanctuary and Wildlife Conservation Trust (CSWCT), Uganda Wildlife Education Center (UWEC), Budongo Forest Conservation Project (BFCP), and the Uganda Wildlife Veterinary Network (UWVN).

MAJOR ACHIEVEMENTS
• Sampled 1,307 wild animals, including 739 nonhuman primates, 142 bats, 365 rodents, and 61 animals of other wildlife taxa.

• Supported the Government of Uganda National Task Force in several disease outbreak investigations, including Ebola virus disease and yellow fever: PREDICT led the wildlife surveillance efforts for these investigations to better understand the role that wildlife may have played in the outbreaks and to identify best strategies for effective prevention, preparedness, and response (see Success Story for more information).
• Installed essential laboratory equipment, supplies, and protocols at Makerere University to enable broad-based testing of hundreds of wildlife samples for viruses of pandemic potential; provided laboratory diagnostic support to other east-central African countries that were conducting disease surveillance in wildlife in order to strengthen diagnostic laboratory capacity regionally.

• Trained 36 wildlife veterinarians and 42 veterinary students in the principles and practice of safe and effective wildlife surveillance.

• Improved facilities and acquired equipment for the safe and secure transport and storage of biological samples collected from wildlife.

• Conducted systematic sampling of wildlife across a land-use gradient in the Bwindi-Mgahinga Conservation Area (as part of the “Deep Forest study”), which was designed to test theories regarding how landscape disturbance influences the emergence of wildlife pathogens in people.

• Introduced a novel, cell phone-based system that assists the Uganda Wildlife Authority with reporting on animal morbidity and mortality events in the Queen Elizabeth Conservation Area.

SUCCESS STORIES

Establishment of One Health Approach to Outbreak Response

PREDICT supported the Uganda National Task Force for Epidemic Preparedness and Response in its investigations of several disease outbreaks to better understand the role that wildlife may play in these events, and to learn lessons for prevention, preparedness, and response for the future. PREDICT-Uganda played an integral role in three important disease outbreak investigations, including Ebola virus and yellow fever (see below for information on disease outbreak response and preparedness), prioritizing sites for wild animal sampling activities in the field and collecting epidemiologic data on potential human risk factors, such as recent movements, hunting activities, and contact with and consumption of sick animals. The National Task Force now applies a One Health approach to disease outbreak investigation, control, and prevention in Uganda by incorporating wildlife investigations into disease outbreak response planning.
Advancement of Wildlife Disease Surveillance and Diagnostics

PREDICT worked closely with the Uganda Wildlife Authority, Makerere University, and the Makerere University Walter Reed Project (MUWRP) to improve infrastructure and systems for detecting pathogens in wildlife. Specifically, PREDICT piloted a cell phone-based monitoring system for reporting wildlife mortality events, which is now integrated into UWA’s ranger-based surveillance program in Queen Elizabeth Conservation Area. In addition, PREDICT established facilities for wildlife disease surveillance at COVAB and equipped MUWRP with equipment and protocols that enabled the laboratory to safely handle and test hundreds of samples from wildlife. Because the MUWRP laboratory is closely partnered with the Uganda Virus Research Institute (UVRI), human and wildlife disease investigations are now better linked as a result of the PREDICT effort.

CAPACITY BUILDING

Diagnostic Laboratory Capacity

PREDICT installed laboratory equipment and provided support and supplies for applying advanced molecular techniques to wildlife samples at the Makerere University Walter Reed Project (MUWRP) Influenza Research Laboratory. PREDICT implemented diagnostic testing protocols for nucleic acid extraction, amplification by polymerase chain reaction, and genetic analysis for viral genera/families known to cause illness, epidemics, and pandemics in people. Sequencing of positive PCR products and characterization of previously unreported viruses was performed at the University of California Davis and the Mailman School of Public Health, Columbia University, New York, both institutions located in the USA.

Training

PREDICT-Uganda personnel were trained on biosafety and PPE use; safe wildlife capture and handling; rodent, bat, nonhuman primate, and bushmeat sampling; sample handling, storage, packaging, and shipping; and laboratory safety. PREDICT provided the same training opportunities to 36 government and nongovernment veterinarians from Uganda Wildlife Authority, Makerere University Wildlife Department, Uganda Wildlife Education Center, Budongo Forest Conservation Project, Kibale Eco-health Project, and to 42 veterinary students at the Makerere University Wildlife Department, with a focus on basic principles of safe wildlife surveillance; personal protection; wildlife sampling; and biological sample collection, transportation, and preservation.

SURVEILLANCE

PREDICT sampled 1,307 free-ranging, captive, confiscated, and depredated wildlife (739 nonhuman primates, 365 rodents, 142 bats, and 22 bovids). PREDICT Country Coordinator Dr. Benard Ssebide (left), PREDICT field veterinarian Dr. Racheal Mbabazi (standing), and Uganda Wildlife Authority rangers collecting samples from a kob exhibiting signs of illness in Queen Elizabeth National Park. This kob was diagnosed with bovine tuberculosis at postmortem, which was confirmed by culture and PCR.
61 animals from other wildlife taxa at high-risk disease transmission interfaces across Uganda, in places where wildlife are likely to have significant interactions with domestic animals and humans (Figures 1 and 2). Surveillance efforts were focused on primates, bats, and rodents; however, birds, carnivores, ungulates and domestic livestock were also sampled when relevant to specific interfaces and disease investigation efforts.

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.
Surveillance was targeted at high-risk disease transmission interfaces where people and/or domestic animals come into direct or indirect contact with wildlife as a result of: ecotourism; presence in and around human dwellings and agricultural fields; the wildlife trade; human migration and travel; and increased exploitation of natural resources in areas that were previously uninhabited or very sparsely populated by human communities or where subsistence hunting and gathering activities were conducted in increasingly disturbed habitats (Table 1).

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>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ecotourism and recreational activities</td>
<td>330</td>
<td>42</td>
<td>53</td>
<td>4</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>26</td>
<td>173</td>
<td>76</td>
<td>9</td>
</tr>
<tr>
<td>Hunted wildlife</td>
<td>2</td>
<td>13</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Wildlife preying on livestock or their food</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pristine habitat</td>
<td>10</td>
<td>136</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Wildlife trade</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Wildlife being studied</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protected areas</td>
<td>219</td>
<td>1</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>107</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other high-risk interfaces</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>739</td>
<td>365</td>
<td>142</td>
<td>61</td>
</tr>
</tbody>
</table>

Animal Mortality Monitoring Program (AMMP)

PREDICT piloted a novel cell phone-based animal mortality monitoring program in Queen Elizabeth Conservation Area in collaboration with the Uganda Wildlife Authority. The pilot study was implemented by the Smithsonian Institution in collaboration with UWA and MGVP, Inc.. The aim of the program was to serve as an early warning system for emerging infectious diseases that can pass from animal populations into the human population. Rangers in Queen Elizabeth Conservation Area used Java enabled phones (with GPS functions) during their daily patrol to report any dead or sick wild animals. Authorities were alerted to reports of wildlife morbidity and mortality in real time through the mobile phone based internet technology.

Over 1695 records of sick (n = 831) and dead (n = 864) animals of more than 30 species were reported using this system. Among these reports, 145 cases were identified by rangers as potentially disease-related. Starting in January 2014, a team of PREDICT and UWA veterinarians and researchers responded to reports and collected samples from specific cases for pathogen detection, providing training, supplies, and support to the Pathology Department at Makerere University. Specifically, UWA and the AMMP team responded to 50 field reports of wildlife mortalities and collected samples from 10 cases: eight of these mortalities have been investigated by veterinary pathologists at the time of this publication, and causes of death
included infectious disease, trauma, and suspected toxicity. The mortalities were in proximity to human inhabited areas, and several of the carcasses were butchered and consumed by people in local communities, highlighting the potential for cross-species transmission of pathogens through the shared environment or through direct contact and consumption. The pilot program illustrated that: 1) animal mortality events can be reported in real-time; 2) the cell phone-based methodology is a simple, fast, effective, easily replicable, and inexpensive means to report morbidity and mortality; 3) sample retrieval for laboratory diagnostics is feasible; and 4) when implemented in conjunction with a pathology program, can be an effective means to document and monitor mortality and potential pathogenicity of pathogens in animal hosts.

Deep Forest Study

Land-use change represents one of the most substantial and pervasive threats to biodiversity. Furthermore, land-use change is an important driver of novel disease and zoonotic disease emergence events, as it modifies the risk of cross-species transmission by perturbing the dynamics of pathogens in wildlife hosts and/or by bringing new pathogens and hosts into contact. PREDICT tested theories regarding how wildlife pathogens emerge to infect people by conducting systematic sampling of wildlife across a gradient of human land use in the Bwindi-Mgahinga Conservation Area as part of the global PREDICT Deep Forest study (see Deep Forest section), which was also conducted in Brazil and Malaysia. The aim of the Deep Forest study was to evaluate how increasing land-use development affects: 1) patterns of biodiversity; 2) corresponding patterns of viral diversity; and 3) patterns of human occupancy, abundance, and behavior that may influence contact rates with wildlife in dynamic landscapes.

In Uganda, for the Deep Forest study, we systematically sampled wildlife at eight field sites situated along a gradient from a pristine forest landscape to a semi-disturbed landscape to a highly disturbed, semi-urban landscape. Disturbance was measured at two scales: 1) the landscape scale, calculated from satellite imagery, and 2) the local (site) scale, calculated from transect surveys. Deep Forest biodiversity sampling was focused on three high-risk mammal groups: rodents, bats, and non-human primates. At each site, standardized wildlife surveys were used to characterize local species richness and diversity. From each animal captured within these surveys, blood, saliva, and fecal swab samples were collected, with urine and feces also opportunistically collected.

Each sample was analyzed in the laboratory by consensus PCR for 15 high-risk viral genera/families of interest. A subset of the samples was also analyzed with metagenomic deep sequencing, which allowed the detection of the entire community of viruses within the samples. Field surveys were conducted twice per year at each site (once in the wet season and once in the dry season) to minimize the effect that seasonality might have on the likelihood of detection of host and virus species.
Concurrent with the wildlife sampling, the Deep Forest Human Contact (DFHC) survey was deployed to characterize human-animal contact at each study site. A total of 200 households situated in or immediately adjacent to study sites were surveyed. Particular attention was paid to types and frequencies of contact with bats, rodents, and non-human primates, as well as with other types of wild and domestic animals. The majority of households reported rodents and primates in or around their dwellings, with more than half reporting being bitten or scratched by wildlife (primarily rodents); additionally, some respondents reported that they had eaten wild animal meat in the past.

**DISEASE OUTBREAK RESPONSE AND PREPAREDNESS**

PREDICT assisted the Uganda National Task Force in disease outbreak response during the following outbreak investigations:

**Yellow Fever Outbreak in Northern Uganda (November 2010)**

In November 2010, the Ministry of Health received reports of an undiagnosed illness in northern Uganda. A team of Ministry of Health staff obtained samples from the human cases for testing. Preliminary results from laboratory investigations conducted by the Uganda Virus Research Institute (UVRI), Central Public Health Laboratories (CPHL), and eventually CDC Atlanta were negative for Ebola, Marburg, Crimean-Congo hemorrhagic fever, Rift Valley fever, and bacterial pathogens, such as typhoid.

In response to the need for additional epidemiological and laboratory investigations, the Ministry of Health in collaboration with the Ministry of Agriculture Animal Industry and Fisheries (MAAIF), Makerere University College of Veterinary Medicine, School of Public Health Makerere, WHO, USAID, African Field Epidemiology Network (AFENET), and CDC sent multi-sectoral transdisciplinary teams comprised of physicians, epidemiologists, veterinarians, and environmental health officers to facilitate the extended investigations and initiation of preliminary control measures. The PREDICT team was asked to participate on both the Epidemiology and Surveillance and the Veterinary and Vector subcommittees, providing technical expertise in the area of wildlife surveillance.

The objectives of the investigations were to: 1) conduct extended epidemiological and laboratory investigations to enable adequate description and characterization of the outbreaks; 2) establish systems for case identification, referral, isolation, infection control, and clinical management; 3) conduct an environmental risk assessment to determine the role of environmental exposures in the transmission of the illness under investigation; 4) investigate reports on the ongoing epizootic and determine its zoonotic potential; 5) assess high risk behaviors and knowledge gaps to facilitate development of an appropriate risk communication strategy; and 6) ensure effective coordination of the investigation and response efforts.

Each of the specialized teams conducted community visits to identify cases; interview the cases and/or relatives of the deceased cases; and collect human, environmental, and animal samples. PREDICT used available human epidemiological and clinical case information and worked closely with the CDC officers on-site to prioritize sites for animal sampling activities in the field, including visits to case homes to gather additional epidemiologic information ranging from peoples’ observations and consumption of sick animals, recent movements, involvement in wildlife hunting, and bush meat consumption. In addition, PREDICT sampled animals, primarily rodents and domestic livestock, near homes and villages of affected people. Bushmeat
hunters agreed to cooperate with PREDICT and bring back carcasses for sampling; however, no hunters had successful hunts while the PREDICT team was in the field. The outbreak was eventually confirmed by the CDC as yellow fever in late December.

**Ebola Virus Disease Outbreak in Luwero (May 2011)**

In May 2011, a patient with signs of viral hemorrhagic fever presented to a hospital in the Luwero District and died. Ebola was confirmed through testing performed by UVRI. The Ministry of Health in Uganda established a National Task Force responsible for the outbreak investigation and response. PREDICT assisted the National Task Force through wildlife surveillance efforts aimed at identifying the potential source of the outbreak.

The wildlife surveillance activities were implemented in collaboration with UWA, MAAIF, Ministry of Health, CDC Uganda, and AFENET. PREDICT provided the materials and expertise needed for capture and sampling of potential wildlife hosts, the associated field investigations, and the laboratory analyses conducted at UVRI, MAAIF, and Columbia University. PREDICT sampled 10 vervet monkeys, two bush pigs, and one bushbaby. Viral pathogen testing for arenaviruses, flaviviruses, filoviruses, paramyxoviruses, and coronaviruses was performed, and all samples tested negative.

**Ebola Virus Disease outbreak in Kibale (July 2012)**

In July 2012, Ugandan health officials and WHO reported an outbreak of Ebola virus disease in the Kibale District, Uganda. This was confirmed by laboratory investigations conducted at UVRI, and the virus strain was identified as Sudan virus. The suspected index patient was a 16 year-old female. The Ebola National Task Force established subcommittees to implement outbreak response activities, including an Ecological Studies (ES) subcommittee to initiate and coordinate ecological surveillance in and around the outbreak area.

The ES subcommittee was tasked with: 1) determining if there are any links between the index case and possible animal reservoirs, e.g. monkeys, bats, and other animals; 2) identifying wild animal species that may be natural reservoirs for Ebola virus in the Kibale district; 3) collecting samples to screen for Ebola virus which may be circulating in these wild and domestic animals as reservoir species or as a result of primary or secondary spillover; and 4) characterizing human-animal interactions that may increase opportunities and risk of contracting and transmitting the virus.

PREDICT conducted a human-wildlife contact survey in the areas surrounding the outbreak to characterize human-animal interactions that may have increased opportunities and the risk of contracting and transmitting the viral pathogens. Surveyed households (n=54) reported that rodents, bats, and primates were by far the most common wildlife species involved in human-wildlife contact.
interactions. Most households reported seeing rodents, bats, or primates near their dwellings, with 78% of households reported seeing rodents inside their dwelling and 22% of households seeing both bats and rodents in their dwelling. Households also reported having direct contact with rodents in their home, mainly by being bitten by a live rodent or handling a dead rodent. Nearly half of the households also reported consuming fruit or vegetables that had evidence of being damaged or partially consumed by wild animals, most commonly by rodents, primates, and bats. Based on data obtained through this preliminary survey, there is sufficient contact between humans and bats, nonhuman primates, and rodents to enable transmission and spillover of pathogens commonly shared by these taxonomic groups.

REFERENCES
