excerpts from
Reducing Pandemic Risk, Promoting Global Health

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The Kingdom of Cambodia is located in Southeast Asia, bordering Thailand, Lao PDR, and Vietnam and spans 181,035 km² (Kingdom of Cambodia 2010). Currently, the population exceeds 15 million, with a density of 82 people per square kilometer and with 6.3 million Cambodians existing on an income of less than two US Dollars per day. Greater than 30% of the population lives within five kilometers of forest, and forest resources account for 10% to 20% of household consumption supplies (FA 2010). The World Bank reports agriculture and forestry as contributing to nearly 40% of the country’s Gross Domestic Product (GDP).

Cambodia is rich in biodiversity and forms part of the Indo-Burma biodiversity hotspot, having a high number of endemic species that are also threatened by the loss of a large proportion of their original habitat (Myers et al. 2000). Nearly 59% of Cambodia is still forested and includes evergreen, semi-evergreen, deciduous, swamp, mangroves, and bamboo forest. In the center of Cambodia is the ecologically rich Tonle Sap Lake that was named a UNESCO biosphere in 1997. Tonle Sap Lake is the largest freshwater lake in Southeast Asia. In the monsoon season, the lake increases significantly in size (Ministry of Environment 2001), and there is concurrent flooding of nearby fields and forests, providing important breeding grounds for fish and wetlands that provide habitat to migratory birds and a large variety of other wildlife.

Despite being listed as the 13th most forested country in the world (by percent land cover; FAO 2011), Cambodia’s forests have been severely degraded over the past two decades as a result of logging, forest fires, intensified shifting cultivation, land-grabbing, and encroachment. Recent research has revealed that on a national scale, Cambodia was one of the worst three countries in terms of forest loss, with a loss of 7.1% of the country’s forests from 2000-2012 (Hansen et al. 2013). Rapid economic development and granting of large land concessions for the combination of rural poverty and the high demand for wild animal products locally and internationally, results in Cambodia being a key source, consumer, and conduit for wildlife trade.
industrial agriculture and extractive industry have led to dramatic deforestation and large-scale land-use change. Few areas of Cambodia’s forest remain untouched by anthropogenic disturbance (Figure 1). Such agricultural intensification and land-use change are key drivers for emerging diseases (Jones et al. 2008; Jones et al. 2013; MacFarlane et al. 2013), making Cambodia an important country of focus for disease surveillance at the increasing number of wildlife-livestock-human interfaces.

Figure 1. Map showing human disturbance of land across Cambodia.

There are currently 27 Protected Areas and 10 Protection Forests in Cambodia, representing about 24% of the country’s total land area (UNEP-WCMC 2014). Existing forests provide a wealth of opportunities for wild game hunting, and the combination of rural poverty and the high demand for wild animal products locally and internationally (i.e. pet trade, food and traditional medicinal purposes), combined with porous international borders, results in Cambodia being a key source, consumer, and conduit for wildlife trade. Since 2001, the Forestry Administration’s Wildlife Rapid Rescue Team has confiscated over 56,000 animals from the illegal wildlife trade in Cambodia (Wildlife Alliance 2014).

Bats and rodents are consumed and traded widely within Cambodia, and hunted nonhuman primates are traded for medical laboratory testing (macaques), traditional medicine (lorises), human consumption, and as pets (WCS, Cambodia unpublished). Seasonal trade results in over three tons of rats exiting Cambodia per day for consumption in Vietnam (Phnom Penh Post 2012). The scale and extent of global wildlife trade has expanded over the past decade. Increased purchasing power and globalization have led to a rising demand for exotic animals, many
sourced from the wild, in both developed and developing nations (Nijman et al. 2012). Over 35 million CITES-listed animals were exported from Southeast Asian countries from 1998 to 2007 (Nijman 2010), and almost 30 million of these represented wild-caught individuals. These figures exclude all underground, local, and informal trade.

Since the discovery of SARS coronavirus in wildlife in Asian markets (Bell 2004), the wildlife trade is increasingly recognized as a potential source of zoonotic pathogen spillover and spread in human populations (Edmunds et al. 2011; Karesh et al. 2005; Pavlin et al. 2009; Smith et al. 2012; Swift et al. 2007). The scale of Cambodia’s illegal wildlife trade makes it a high-risk country for the spillover of emerging pathogens of wildlife-origin into humans and other animals. Early detection of potential zoonotic pathogens in wildlife is therefore important for prevention of emerging zoonoses in Cambodia and neighboring countries partnering in the wildlife trade. In Cambodia, PREDICT investigated the implications of human and wildlife interactions on risk of pathogen emergence, with a special focus on the wildlife trade.

Cambodia has recorded 56 cases of H5N1 Highly Pathogenic Avian Influenza infection in humans since 2005 and nine cases in 2014 as of March 17th (ProMED 2014). Most deaths have been linked to disease outbreaks in domestic poultry. With a history of inter-ministerial collaboration to respond to and combat the threat of avian influenza, the Royal Government of Cambodia recognizes the importance of a One Health approach to zoonotic disease surveillance, response, and prevention and the need to educate the next generation of animal and human health professionals in this cross-sectoral discipline.

PARTNERS
The PREDICT project was implemented in Cambodia through the collaborative efforts of Wildlife Conservation Society and the Pasteur Institute of Cambodia (IPC) to strengthen local capacity to conduct wildlife disease surveillance and to characterize zoonotic disease risk at wildlife-human interfaces. Other partners included USAID and:

Royal Government of Cambodia (RGC):
- Ministry of Agriculture, Forestry and Fisheries (MAFF)
- National Veterinary Research Institute (NaVRI)
- Forestry Administration (FA)
- Ministry of Health (MoH)
- Communicable Disease Control Department (CDC)
- Royal University of Agriculture (RUA)
- Prek Leap National College of Agriculture (PNCA)
MAJOR ACHIEVEMENTS

• Partnered with the Emerging Pandemic Threats PREVENT project to conduct a survey on wildlife hunting and consumption in several villages across three provinces. The aim of the survey was to assess zoonotic disease risk and design interventions targeted at high-risk behaviors (see Success Stories for more information).

• Institutionalized a One Health approach to surveillance, illustrated by a high-level of collaboration between government ministries and PREDICT, including agreements with the NaVRI and FA for their staff to join PREDICT field surveillance across 17 provinces (see Success Stories for more information).

• Established Khmer capacity for wildlife disease surveillance in the government and pre-service veterinary community through training of over 300 personnel (33% women) in PREDICT protocols and proper use of personal protective equipment (PPE). Collaboration among partners led to successful surveillance efforts across a variety of high-risk disease transmission interfaces from consumption of hunted wildlife in villages, markets, and restaurants to contact with wildlife in farms, tourist and religious sites, and rescue centers.

• In partnership with the IPC, PREDICT built in-country capacity for consensus (viral genus/family level) PCR and next-generation sequencing and provided training for NaVRI government laboratory technicians to ensure sustainability of the genomic tools.

• Engaged rangers and hunters from Khmer and ethnic minority villages in sampling and zoonotic disease risk determination activities across the country. PREDICT conducted 14 follow-up meetings in 11 provinces with communities involved in surveillance efforts to present project findings and discuss the risks of zoonotic diseases from wildlife. A total of 165 men and 248 women participated in the meetings. Local authorities concluded the meetings by advising villagers to minimize contact with wild animals through reducing high-risk hunting and trade activities and to improve their meat hygiene practices in order to reduce the risk of contracting diseases from wildlife.

• Participated as an invited member of the Zoonotic Technical Working Group, which includes RGC animal and human health sectors, WHO, and FAO. PREDICT contributed to the creation of the Strategic Plan for Zoonoses Control in Cambodia and worked with the RGC to move towards instituting a surveillance program for animals confiscated from the illegal wildlife trade.

• Integrated PREDICT into the higher-education community in Cambodia through participation of PNCA and RUA veterinary students in field surveillance activities and contributions to development of the new RUA veterinary doctorate degree curriculum.

• Test results from over 7,000 samples from 3,185 animals were presented to the Royal Government of Cambodia and approved for release to the public domain on PREDICT’s HealthMap platform.
SUCCESS STORIES

Understanding Wildlife Consumption and Zoonoses Risk Perception in Khmer Communities

PREDICT and Emerging Pandemic Threats project, PREVENT, conducted a survey of wildlife hunting and consumption in Cambodia to assess potential risk of zoonotic disease spillover into people who participate in these activities. The survey was administered in nine villages across three provinces, concurrent with sampling of hunted wildlife. Hunting and consumption of wildlife are common throughout rural Cambodia, where many isolated, low-income communities still rely on wild animal meat as an important source of nutrition. Key animal groups identified as harboring the greatest proportion of zoonotic viruses (e.g. bats, nonhuman primates, and rodents) are still widely consumed in many villages. This survey aimed to quantify contact between wildlife and people and to begin to identify strategies to reduce the risk of spillover of zoonotic pathogens from wildlife into people since in the rural communities, minimal information is currently available to quantify seasonal household hunting or changes in the abundance and availability of wildlife.

Brief initial household surveys were conducted to obtain information about meat consumption and associated hunting and butchering activities to determine how potential risk associated with these activities was distributed among the population. Results of the household surveys were used to identify two groups of households:

1. Households who hunted and consumed the greatest number of animals in the previous month were invited to participate in the PREDICT sampling study and a quarterly follow-up survey about their domestic animals and consumption of meat; and

2. Comparison households, selected randomly from the other households in the village who agreed to participate in follow-up interviews, were invited to participate in the quarterly survey.

The surveys and surveillance were conducted in ethnic minority and Khmer villages in protected forest areas of Preah Vihear Province in northern Cambodia, Banteay Meanchey Province in Northwestern Cambodia, and in Mondulkiri Province in eastern Cambodia. Follow up quarterly surveys captured seasonal variation. These surveys identified key occupational and behavioral risks within the villages to target for PREDICT surveillance. In some provinces, over 8% of households consumed nonhuman primates, despite living within protected areas where promotion of the illegality of such activities was widespread and such consumption was not traditionally thought to be part of the local culture.

The study identified many reasons why people chose not to consume certain species of wildlife, including fear of disease, indicating some potential to influence and reduce local consumption through heightened awareness of health risks. However, such reasoning was observed to be very personal and not witnessed at an ethnic or community level, thus risk-awareness behavioral change approaches would likely be insufficient. Seasonality in hunting activities was observed, with many households turning to agricultural activities when the climate and landscape allowed, suggesting a preference to cease hunting when alternative foods and improved income sources were available.

The survey process allowed the team to build strong relationships with communities in villages within or bordering protected areas, many of which are now facing the prospect of large-scale local deforestation at recently granted Economic Land Concession sites. Continued surveillance
at these sites has great potential to capture information on the effect of land-use change on hunting patterns and pathogen emergence in wildlife and people.

Building a One Health Network for Surveillance of Zoonotic Pathogens in Wildlife

PREDICT worked closely with the RGC to build capacity for wildlife disease surveillance. The team in Cambodia held a workshop that brought together, for the first time, central and provincial staff from the Ministry of Agriculture, Forestry and Fisheries, MoH, Ministry of Environment (MoE), and higher education authorities. The workshop provided training on emerging zoonoses and surveillance in wildlife. The goals of this workshop were to highlight the cross-sectoral nature of this work and open communications for a One Health approach to collaboration across ministries in future wildlife disease surveillance efforts.

This workshop led to further training requests from the national veterinary schools and ranger teams working with confiscated wildlife. Additionally, the government recognized the importance of adopting the One Health approach, and PREDICT was invited to join the Zoonotic Technical Working Group meetings to bring a wildlife perspective to the discussions. Our field surveillance and diagnostics were a collaborative effort with technical staff members from the government’s National Veterinary Research Institute and Forestry Administration as well as veterinary students who will become the future work force for disease response and surveillance.

CAPACITY BUILDING

Training

PREDICT provided training to a wide range of participants in order to build sustainable capacity for wildlife disease surveillance in Cambodia. Over 100 local partners were trained on core safety; wildlife capture and sampling; data collection and management; rodent, bat, and nonhuman primate identification; and proper use of PPE. In addition, training on wildlife zoonoses, safety, PPE, and sampling was conducted for rangers working closely with confiscated high-risk wildlife species (i.e. nonhuman primates, rodents, and bats). Furthermore, PREDICT held a workshop that brought together central and provincial staff from the MAFF, MoH, MoE, and higher education authorities for training on emerging zoonotic pathogens and wildlife disease surveillance and to promote collaboration across ministries.

PREDICT provided training to the next generation of Khmer veterinarians. A total of 187 students from PNCA and the RUA were educated on wildlife zoonoses. Twenty-two of these veterinary students also participated in field surveillance activities. PREDICT supported the completion of two veterinary thesis projects on nonhuman primate zoonoses, in which the students received training on field surveillance and laboratory analyses. In addition, RUA invited participation by PREDICT in a One Health workshop, which included a presentation on our surveillance strategy. Furthermore, RUA requested that PREDICT contribute to development of the new veterinary doctorate degree curriculum.
Khmer language translations of the comprehensive PREDICT protocols were produced and disseminated to local partners, students, and ministries. Government staff and veterinary students have used the protocols to enhance wildlife disease surveillance in Cambodia through improved safety for humans and animals; heightened awareness of biosafety and ethical issues regarding both the treatment and handling of a variety of animal taxa during trapping, sample collection, or transportation; involvement of animals and humans in research; more appropriate sample and media selection; sample collection, storage, and handling techniques in the field and laboratory; and relevant data collection and management. Technical staff members from the NaVRI and the FA and veterinary students from the RUA and PNCA collaborated on wildlife disease surveillance activities.

**Laboratory Capacity**

PREDICT partnered with the IPC to introduce viral family PCR screening tools and next generation sequencing to Cambodia. PREDICT provided support for equipment and training to NaVRI government laboratory technicians at the IPC to ensure cross-sectoral sustainability of the diagnostic capacity.

**SURVEILLANCE**

Surveillance was conducted across 17 provinces covering all regions of the country and at 82 sites ranging from pristine protected forests to urban markets in populous areas (Figure 2). Country-wide surveys early in the project identified numerous interfaces that pose a high risk of spillover of pathogens from wild animals to humans. In addition, sampling at novel wildlife-human interfaces with high risk for disease transmission continued as the project progressed. The variety of interfaces that were targeted for PREDICT surveillance included (Table 1):

1. Wildlife hunting by ethnic minority and Khmer communities within and outside of protected areas;
2. Wildlife trade by middle-level traders operating within and outside of Cambodia;
3. Crop raiding by wild animals such as rats, wild pigs, and bats;
4. Zoos and wildlife rescue centers (e.g. Angkor Centre for Conservation and Biodiversity, the government wildlife rescue center at the public Phnom Tamao Zoo, and a private zoo where carnivores were fed macaques that had died on Khmer macaque farms);
5. Markets and restaurants selling wildlife (e.g. bat restaurants and markets selling primates for traditional Khmer medicine);
6. Bat guano farms where workers collect feces from under bat roosts for agricultural use;
7. Religious and ecotourism sites such as the Angkor Wat temple area, where macaques have been fed by the public and are now abundant and aggressive, and fruit bats roost amongst the temples and are sometimes targeted for hunting; and
8. Rodents in and around human dwellings and from the Mekong Delta region, traded to Vietnam for food.
Figure 2. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.

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>99</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ecotourism and recreational activities</td>
<td>41</td>
<td>10</td>
<td>96</td>
<td>22</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>51</td>
<td>34</td>
<td>1041</td>
<td>0</td>
</tr>
<tr>
<td>Hunted wildlife</td>
<td>114</td>
<td>344</td>
<td>1017</td>
<td>394</td>
</tr>
<tr>
<td>Markets</td>
<td>54</td>
<td>11</td>
<td>0</td>
<td>115</td>
</tr>
<tr>
<td>Restaurants</td>
<td>0</td>
<td>0</td>
<td>347</td>
<td>0</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pristine habitat</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Other high-risk interfaces</td>
<td>0</td>
<td>54</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>300</strong></td>
<td><strong>552</strong></td>
<td><strong>2503</strong></td>
<td><strong>532</strong></td>
</tr>
</tbody>
</table>
A total of 3,887 animals were sampled including 300 nonhuman primates, 552 rodents, and 2,503 bats. Additional animal groups sampled included those hunted, consumed, and traded commonly by local people, such as wild pigs, deer, and civets (Figure 3). Polymerase Chain Reaction (PCR) screening for 16 viral families and additional pathogens of regional concern was conducted at IPC. The laboratory diagnostic testing occurred concurrent with training of local government veterinary laboratory counterparts, and in some cases veterinary students, to ensure sustainability of the diagnostic techniques in Cambodia.

The following viral families were targeted for testing: arenaviruses, alphaviruses, astroviruses, bocaviruses, bunyaviruses, coronaviruses, enteroviruses, filoviruses, flaviviruses, influenza viruses, hantaviruses, henipaviruses, herpesviruses, orthopoxviruses, paramyxoviruses, rhabdoviruses, lyssaviruses, retroviruses (Lentivirus genus), simian foamy viruses, and seadornaviruses. In addition to optimizing viral screening protocols for wildlife samples and the introduction of two PREDICT universal controls to Cambodia, we initiated next generation sequencing at IPC for pathogen discovery.

**DISEASE OUTBREAK RESPONSE AND PREPAREDNESS**

The focus on PREDICT surveillance concurrent with building of One Health capacity within the Royal Government of Cambodia and the future veterinary workforce has resulted in increased local technical capabilities and cross-sectoral communication to better respond to disease outbreaks involving wildlife.
REFERENCES


