excerpts from
Reducing Pandemic Risk, Promoting Global Health

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Approximately 75% of emerging zoonotic diseases originate in wildlife (Jones et al. 2008). Activities that bring wildlife into close proximity with humans and livestock, such as wildlife trade, expanding human populations, and forest clearance, facilitate the opportunity for pathogen transmission. Wildlife trade has contributed to the emergence of a suite of diseases including SARS, monkeypox virus, and H5N1 influenza A (Guarner et al. 2004; Van Borm et al. 2005). Markets selling wildlife have acted as a source of previous disease outbreaks. In 2003, the SARS outbreak originated in a wildlife market in China and spread to 29 countries on five continents, leading to 8,098 human cases and 774 deaths worldwide and an estimated cost to China’s economy of $16.8 billion in lost tourism (Hai et al. 2004; Lam et al. 2003). Thus, wildlife trade and zoonotic diseases pose significant threats to human health, economies, and biodiversity in Southeast Asia.

Lao PDR

Lao People’s Democratic Republic (PDR) is a landlocked country in Southeast Asia with a population of 6,288,000 and a relatively low population density (27 people/km²) for this region (UN 2014). In 2002, the forest cover in Lao PDR was estimated at 41% (Bourgeois Luthi 2012), and there are currently 23 National Biodiversity Conservation Areas (NBCAs) and two corridors in Lao PDR, covering 3.5 million ha, which is equivalent to almost 15% of the country’s land area (4th NRCBD report). Due to the country’s low population and extensive forest cover, it is home to a rich diversity of wildlife, including mammal, reptile, bird, and amphibian species of national or global importance (Duckworth 1999).

Lao PDR is one of the poorest countries in which chronic malnutrition is high (Phengxay et al. 2007). Among non-Lao Tai ethnic groups, about 60% of children under five years of age are...
stunted, while 41% of children younger than five and 64% of children younger than two years of age suffer from anemia. Many also suffer from sub-clinical Vitamin A deficiency (Johnson et al., 2010; DOS and UNICEF 2008). In ethnic groups living in remote areas, wildlife can play a crucial household food source, and food security can be threatened by wildlife declines (Johnson et al., 2010). Thus, sustainably managing wildlife is important to both protect Lao PDR biodiversity and to reduce poverty and malnutrition in remote areas.

However, nearly all wildlife species are declining, and many are already at alarmingly low levels (Duckworth 1999; Johnson et al. 2010). Populations are increasingly threatened by high levels of hunting for domestic and international wildlife trade, as well as habitat loss, driven by shifting cultivation, logging for timber, and conversion of natural forests to cash crops and plantations at an unprecedented rate (Johnson et al. 2010; Duckworth 1999). Domestic wildlife trade in wet markets started in the 1980s and gained momentum after 1986, with the economic opening of the country following the inception of the New Economic Mechanism (Bourgeois Luthi 2012). Despite Lao PDR law prohibiting wildlife trade (Wildlife and Aquatic Law 2007), trade is common with wildlife being primarily sold for food and to a lesser degree for traditional medicine, pets, and for ornaments. The market availability of wildlife is also increasing due to the growing accessibility of wildlife areas resulting from road and infrastructure development and the opening up of wildlife areas due to illegal logging and other new activities (TRAFFIC 2008). The impacts of trade have been widespread, with large-sized mammals decreasing from the early 1980s and small mammals declining since the 2000s (Bourgeois Luthi 2012).

PREDICT and its partners in Lao PDR (i.e. Department of Livestock and Fisheries, including the provincial and district offices of Agriculture and Forestry; National Animal Health Laboratory; and the National Emerging Infectious Disease Coordination Office (NEIDCO)) built on previous partnerships between the human and animal health sectors that were developed during avian influenza outbreaks to expand the wildlife surveillance program and enhance capacity for early detection and response to emerging zoonotic pathogens in Lao PDR.

Wildlife trade in a market where the public come to buy, butcher then picnic on wildlife. Vendors openly sell both live and dead wildlife including flying squirrels, civets, wild birds, hares, and wild pigs.
PARTNERS
PREDICT partners in Lao PDR included the Wildlife Conservation Society (WCS), USAID, and other partners:

• Department of Livestock and Fisheries, including provincial and district offices of Agriculture and Forestry
• National Animal Health Laboratory (NAHL)
• National Emerging Infectious Disease Coordination Office (NEIDCO)

MAJOR ACHIEVEMENTS

• Built national capacity for wildlife disease surveillance and viral detection to enable prompt response in the event of a disease outbreak (see Success Stories for more information).

• Conducted over 376 visits to 96 markets or roadside stalls across 15 provinces of Lao PDR where wildlife volume and species surveys were performed to identify key interfaces where wildlife and humans come into contact.

• Hosted workshops for central and provincial-level government officials from the Ministry of Health and the Ministry of Agriculture and Forestry. The workshops raised awareness of the One Health approach to zoonoses and emerging infectious diseases (EIDs) from wildlife and were attended by 54 provincial government officials.

• NAHL successfully implemented PREDICT’s diagnostic laboratory protocols to test wildlife samples using broadly reactive consensus PCR assays.

• Collected samples from 2,693 wild animals from high-risk human-wildlife disease transmission interfaces across Lao PDR.

• Increased awareness on EIDs of wildlife-origin for 110 government personnel and eight students from the National University of Laos.

• Held One Health meetings with representatives from the central government and 12 provinces of Lao PDR to share results and recommendations for the project. In total, 95 people attended the meetings, including representatives from public health, agriculture and forestry, natural resource and environment, and industry and commerce. In addition, managers from markets that were found to be important wildlife-human interfaces were invited to the meetings.

SUCCESS STORY

Lao PDR Develops the Laboratory Capacity to Respond to Deadly Disease Outbreaks

Through the PREDICT project, Lao PDR developed in-country capacity to help detect wildlife pathogens and respond to potentially deadly disease outbreaks in ways that were not possible before. PREDICT developed innovative techniques for rapid detection and diagnosis of high-risk viral families through the use of synthetic DNA plasmids and broadly reactive consensus polymerase chain reaction (PCR). These techniques are powerful tools in the event of a disease outbreak. They
allow for rapid, broad screening to help rule in or out the involvement of viral pathogens in an outbreak. To develop these capabilities, a gradual and sustained capacity building program was undertaken with staff from the government animal laboratory and NAHL.

In November 2011, three NAHL staff members attended a month-long training at the Pasteur Institute in Cambodia. At this training, NAHL staff received training on personal protective equipment (PPE) use, laboratory biosafety, and protocols to extract DNA and RNA from samples. This capacity development was followed by a second month of training at the Pasteur Institute in June 2012, after which NAHL staff were capable of performing nucleic acid extraction of PREDICT samples.

In October 2012, a UC Davis laboratory expert conducted a one-month training session at NAHL on family level viral PCR for coronaviruses and rhabdoviruses, quality control, and cDNA production to allow shipping of stable testing products. Following this training, NAHL staff performed these protocols with limited ongoing assistance through weekly calls with UC Davis laboratory technical staff.

In-country PREDICT staff provided ongoing training and assistance with database and sample management. The collaboration between NAHL and the Pasteur Institute in Cambodia has allowed continued quality assessment of the PREDICT laboratory work at NAHL, with positive PCR samples sent to the Pasteur Institute for confirmatory testing.

To date, NAHL has performed RNA extractions and produced cDNA on more than 2,500 wildlife samples and conducted PCR on more than 700 samples. The provision of recurrent high quality training and application of techniques with consistent feedback to laboratory personnel has led to sustainable capacity for viral screening at NAHL, which is now prepared to assist in the event of a disease outbreak.

**CAPACITY BUILDING**

**Increasing Awareness of Emerging Infectious Diseases**

In July 2011, PREDICT hosted a workshop for central and provincial level government officials from the Ministry of Health and the Ministry of Agriculture and Forestry to increase awareness of EIDs. Twenty-five participants attended, including representatives from Houaphanh, Xiengkhuang, Vientiane, Bolikhamsay, Khammoune, Champassak, and Attapeu Provinces. The workshop covered a range of topics, including EIDs in wildlife, high-risk interfaces for disease transmission, surveillance for wildlife zoonoses in Lao PDR, risk mapping of high-risk
species and disease transmission interfaces, sample and data collection, wildlife handling and management, and use of PPE. At the end of the workshop, the attendees shared useful comments and information on human-wildlife risk interfaces.

Additional workshops to train greater numbers of provincial employees were requested. In response, PREDICT held three workshops in Bolikhamsay, Xieng Khouang, and Champassak Provinces for 54 provincial governmental officials from the Livestock and Fisheries Division, Forest Conservation Division, and Forest Inspection Division. The workshops raised awareness of the utility of a One Health approach to detection, prevention, and control of zoonoses and EIDs from wildlife. Workshop participants were also provided training on proper use of PPE.

Building on the collaborative work between PREDICT and the Emerging Pandemic Threats PREVENT project to identify high-risk market interfaces in Lao PDR, PREDICT staff were invited to present on wildlife-origin EIDs at market stakeholder meetings in Vientiane and Vang Vieng in order to increase awareness among market owners and provincial and district public health staff regarding the zoonotic disease risk associated with wildlife markets.

Developed National Capacity for Field Surveillance of Wildlife Zoonoses

Training of Trainers. During the course of the PREDICT project, national capacity was significantly increased for wildlife disease surveillance. Two team members received extensive training on wildlife disease surveillance techniques and database management in order to in-turn provide knowledge and tools for national government staff and university students. In addition to education on EIDs and training on field anesthesia and wildlife surveillance methods, staff members also received unique training on wildlife necropsy techniques at the Bronx Zoo, sample bar-code systems, observational wildlife trade survey techniques, human-animal exposure survey techniques, and statistics. The PREDICT staff provided field-based training for NAHL staff on wildlife surveillance techniques and assisted NAHL with management of their sample database and specimen tracking. In addition, they supervised PREDICT student thesis projects on wildlife hunting practices, assisting them with project design and analysis. PREDICT Lao PDR staff also co-presented on EIDs at provincial government workshops, the 2013 Wildlife Health Forum in Bangkok, and the One Health symposium in Lao PDR in 2013.
Training of Government Staff. Over eight months of collaborative field work was performed by staff from PREDICT and NAHL, the government body responsible for animal disease outbreak response. On each field trip, NAHL staff members accompanied the PREDICT team and received field based training on use of PPE, safe wildlife sampling, wildlife species identification, sample labeling, cold chain maintenance for viral samples, and sample transportation. Eight NAHL staff members were trained, and the agency is now capable of conducting wildlife zoonoses surveillance independent of the PREDICT team. PREDICT also supported the participation of NAHL staff in the first regional wildlife pathology workshop in Vietnam, as well as biological safety cabinet and laboratory fume hood training sessions.

Training of University Students. Over three years, eight students from the National University of Laos were trained by the PREDICT team on observational survey methods, PPE, wildlife sampling techniques, and sample storage. PREDICT then supported these students in their efforts to conduct research in wildlife hunting villages for their thesis projects. In addition to training the next generation of scientists, valuable information was obtained on wildlife hunting and consumption practices, which can be used for zoonotic risk mitigation strategies.

Established Diagnostic Capacity at the National Animal Health Laboratory for Viral Family Testing on Wildlife Samples

Capacity was developed at NAHL for testing viral families using synthetic DNA plasmids and broadly reactive consensus PCR assays. Multiple training sessions at Pasteur Institute of Cambodia and an in-house session provided by UC Davis laboratory personnel has allowed NAHL personnel to independently conduct RNA extraction, cDNA production, viral family level PCR for coronaviruses and rhabdoviruses, and quality control assessments.

SURVEILLANCE

Samples have been collected from priority taxonomic wildlife groups (i.e. bats, nonhuman primates, and rodents) at high-risk human-wildlife disease transmission interfaces across 14 provinces of Lao PDR, focusing on the wildlife trade (i.e. markets and roadside stalls selling wildlife), subsistence hunting in indigenous villages, captive wildlife collections in zoos or held...
as private pets, wildlife in and around human dwellings, and ecotourism sites (Figure 1). A total of 2,693 animals (1,530 rodents, 956 bats, 45 primates, and 162 from other species, including civets) were sampled from these high-risk interfaces (Figure 2 and Table 1). Samples were screened using consensus PCR for alphaviruses, arenaviruses, astroviruses, coronavirus, filoviruses, flaviviruses, hantaviruses, henipaviruses, herpesviruses, influenza viruses, rhabdoviruses, lyssaviruses, seadornaviruses, paramyxoviruses, poxviruses, bunyaviruses, and retroviruses.

Figure 1. 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>Ecotourism and recreational activities</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hunted Wildlife</td>
<td>1</td>
<td>408</td>
<td>125</td>
<td>22</td>
</tr>
<tr>
<td>Markets</td>
<td>20</td>
<td>1118</td>
<td>831</td>
<td>138</td>
</tr>
<tr>
<td>Private sale</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Zoo and sanctuaries</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>45</strong></td>
<td><strong>1530</strong></td>
<td><strong>956</strong></td>
<td><strong>162</strong></td>
</tr>
</tbody>
</table>

**Market Surveys**

Over a 3.5 year period, 376 market surveys were conducted, each survey consisting of one visit to a market or roadside stall. Two field staff were trained in observational market survey techniques. At each market or roadside stall, surveyors recorded the GPS position, the species of animals for sale, the body part type being sold, the number of carcasses or estimated weight of body parts being sold, the condition of the item (live, dried, fermented, fresh, frozen, pickled, or smoked), and sale price.

Detailed biosecurity and shopper origin surveys were conducted in collaboration with PREVENT over a three-month period. These more detailed surveys were conducted once per market, and general characteristics of the market were recorded, such as license plates observed on vehicles near the market (to assess shopper origin), observation of foreigners shopping in the market, volume of domestic animals, and general hygiene throughout the market. Observations on wildlife vendor and butcher hygiene was limited to observation of one wildlife vendor and one wildlife butcher (if present) per day for 30 minutes.
Characterization of the Wildlife Trade

Through work undertaken by PREDICT, we now have a better understanding of the scale of the wildlife-human contact interface in markets. Over 376 visits to 93 markets or roadside stalls during the PREDICT project, 17,756 wild birds, 11,554 mammals, and 448 reptiles were observed. Mammal observations included 6,862 rodents, 3,638 bats, 58 primates, and 2,151 kg of ungulates. These results represent a fraction of wildlife trade in Lao PDR and demonstrated the high level of human exposure to wildlife that is occurring. Overall, trends showed that freshly killed wildlife was more commonly sold compared to live wildlife (30% of rodents, 10% of wild birds, 4% of bats, and 53% of primates observed at sale were alive). However at certain markets, the number of live animals at the time of sale was relatively high. For example, of the 4,526 wild animals observed during sale over five visits to a market in southern Lao PDR, 85% of the individuals were alive (mostly rodents and lizards). In addition, at a market on the main northern transport route, of the 677 wild animals observed, 82% were sold alive (mostly wild birds).

Biosecurity surveys in markets undertaken by the PREDICT team in collaboration with PREVENT, highlighted the occurrence of high-risk behaviors for pathogen contamination, such as vendor butchering of wildlife combined with absence of basic sanitary measures (i.e. hand washing and table cleaning). Buyers at markets were not only locals, but also persons from other provinces and other countries. For example, customers at the market in southern Lao PDR often came from Thailand to purchase, butcher, and consume wildlife. In addition, the demand for wildlife in Lao PDR by urban populations as a luxury food item is widespread (Singh 2008; pers comm. Lao PDR government officials). Therefore, as wildlife is increasingly purchased and transported to densely populated urban locations to meet this demand, the risk of pathogen spread to wider populations increases.

During sampling trips by the PREDICT team in rural Lao PDR hunting communities, information was gathered on high-risk behaviors, including wildlife butchering with poor biosecurity practices, consumption of large numbers of rodents and bats in areas where larger wildlife has been overharvested, and consumption of raw or partially cooked wildlife, including nonhuman primates. Furthermore, hunting villages also kept primates alive at the village prior to sale or consumption.
REFERENCES


