excerpts from
Reducing Pandemic Risk, Promoting Global Health

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Countries in Southeast Asia rank among the highest in the world for biodiversity yet have experienced some of the most rapid deforestation. Over the past few decades, oil palm has been one of the most rapidly expanding crops in the region (Koh and Wilcove 2008). Malaysia is the second largest oil palm producer in the world, and much of the deforestation in the country has occurred as a result of land conversion for palm oil plantations. Anthropogenic land-use change, including the expansion and intensification of livestock production, has brought human settlements and commercial agriculture into proximity with wildlife. In Malaysia’s villages, there are few or no barriers between people, their domestic animals, and wildlife.

Malaysia has been identified as a hotspot for zoonotic disease emergence and is where Nipah virus, a deadly paramyxovirus carried by pteropid fruit bats, first emerged in 1998 (Chua 2003; Halpin et al. 2011). The first emergence of Nipah virus occurred on a large-scale pig farm on the outskirts of Malaysia’s fifth largest city Ipoh in the state of Perak approximately 200 km north of the capital Kuala Lumpur. The intensification of swine production, coupled with the presence of cultivated fruit orchards on the farm, allowed for the spillover of Nipah virus from bats into pigs and humans. Its subsequent circulation ignited the epidemic that ultimately killed nearly half of the 200 infected farm workers and had a devastating impact on the swine production industry in Malaysia (Pulliam et al. 2012). The high degree of contact among people, livestock, and wildlife in Malaysia contributes to its shared status among countries highly vulnerable to zoonotic disease emergence, and therefore Malaysia is a country prioritized for development of a coordinated national wildlife disease surveillance system (Jones et al. 2008).

Because of the cross-sectoral impact of zoonotic diseases involving wildlife, livestock, and people (e.g. Nipah virus, HPAI H5N1, and Middle East Respiratory Syndrome (MERS) Coronavirus),
prevention and control of zoonotic pathogens can be substantially enhanced by the coordination of efforts from ministries of health, agriculture, and environment (the wildlife authority). The utility of a One Health approach for infectious disease research, prevention, and control is increasingly recognized among scientists and some government agencies globally, but divisions among ministries persist and make coordinated surveillance activities difficult to realize. Malaysia has had firsthand experience responding to a novel zoonotic disease outbreak. During the 1998 Nipah outbreak, the Malaysian Government established the Inter-Ministerial Committee for Zoonotic Disease Control. This inter-ministerial platform was an important factor in controlling the Nipah virus outbreak, which required close coordination among human and animal health agencies. However, in the years following the Nipah outbreak, the Inter-Ministerial Committee for Zoonotic Disease Control stopped meeting, and there was no regular communication among livestock, wildlife, and health departments outside of emergencies.

In 2009 and 2010, PREDICT began to discuss the need to re-establish this inter-ministerial platform to facilitate the integration of wildlife disease surveillance into the public health infrastructure. In part due to PREDICT’s efforts, and in recognition of the growing importance of the One Health concept, the Inter-Ministerial Committee for Zoonotic Disease Control began to meet regularly again. In addition to government partners, local universities involved in zoonotic disease surveillance are invited to attend and share their research. PREDICT attended several of these meetings to inform members of the committee of our progress.

In 2012, PREDICT, the Ministry of Health, the Department of Veterinary Services, and the Department of Wildlife and National Parks also established the Zoonosis Technical Working Committee. This cross-sectoral network convenes to deal with human and animal health issues, including mitigation strategies for decreasing the risk of emerging infectious diseases, and reports to the Inter-Ministerial Committee for Zoonotic Disease Control. PREDICT also increased capacity within a laboratory network that exchanged sample testing techniques, technologies, and personnel to enhance disease detection and wildlife surveillance capacities in Malaysia. These platforms enhanced the Government of Malaysia’s ability to detect and respond to zoonotic pathogens.

**Partners**

PREDICT partners in Malaysia included EcoHealth Alliance (EHA), the Center for Infection and Immunity (CII) at Columbia University, Global Viral, USAID, and the Smithsonian Institution.

Malaysian partners included:

- Department of Wildlife and National Parks (DWNP)
- Department of Veterinary Services (DVS)
- Veterinary Research Institute (VRI)
- Ministry of Health (MoH)
• Department of State Health Sabah (DSHS)
• Sabah Wildlife Department (SWD)
• Danau Girang Field Centre (DGFC)

MAJOR ACHIEVEMENTS
• Expanded the One Health Workforce by forming the Zoonosis Technical Working Committee with the Department of Veterinary Services (DVS), Ministry of Health (MoH), and the Department of Wildlife and National Parks (DWNP; see Success Stories for more information).

• Trained 21 DWNP officers from states where PREDICT sampling was conducted. PREDICT and DWNP sampled 1,063 animals in Peninsular Malaysia.

• Helped DWNP establish a dedicated surveillance team to conduct routine surveillance and respond to outbreaks. The DWNP-dedicated surveillance team independently sampled an additional 423 animals for PREDICT and another 313 outside of the PREDICT project in Peninsular Malaysia following PREDICT protocols.

• Provided good laboratory practices and biosafety training to 45 staff from DWNP, VRI, and SWD. In total, PREDICT trained 130 individuals from government partners, local universities, and NGOs in surveillance and diagnostic techniques including sharing protocols.

• Surveyed priority wildlife taxa within pristine, semi-disturbed (agricultural), and disturbed landscapes along the lower Kinabatangan River during the wet and dry seasons for the Deep Forest study.

• Coordinated with the Department of State Health Sabah and Sabah Wildlife Department (SWD) to conduct behavioral surveys to measure and characterize human-wildlife contact at the Deep Forest sites in Sabah. PREDICT surveyed 406 villagers, oil palm workers, and tourists.

• Created the Wildlife Health Unit, a dedicated surveillance team, with Sabah Wildlife Department. The Wildlife Health Unit sampled 1,179 animals in Sabah from 2012 to 2014.

• Helped create BSL-2 molecular diagnostic laboratories at DWNP, VRI, and the Wildlife Health Unit in Sabah for livestock and wildlife pathogen testing.

• PREDICT and the MoH screened 136 archived samples from Orang Asli community (indigenous population) patients with acute febrile illness at the National Public Health Laboratory (NPHL) using PREDICT PCR universal controls and viral family protocols.

• Tested 8,324 samples from 2,665 animals from Peninsular Malaysia and Sabah at DWNP, VRI, WHGFL, and CII using PREDICT PCR and other protocols.
SUCCESS STORY

Expanding the One Health Workforce

Although Malaysia is considered a hotspot for zoonotic disease emergence, infrastructure for detecting novel and potentially zoonotic wildlife pathogens was largely lacking in-country prior to implementation of the PREDICT project. In addition, since the 1998 Nipah virus outbreak there had been little collaboration in Malaysia across sectors with expertise in human, domestic animal, and wildlife health; ecology; and laboratory sciences. While the utility of a One Health approach for infectious disease research, prevention, and control is increasingly recognized among scientists and some government agencies globally, divisions among ministries persist, making coordinated surveillance activities challenging.

One of PREDICT-Malaysia’s first tasks was to help the Malaysian Government recognize the importance of the One Health concept and the need for regular communication among the human, wildlife, and livestock sectors. Using Malaysia’s experiences with Nipah virus as an example, PREDICT-Malaysia was able to highlight the need for regular cross-sector communication, which resulted in the re-establishment of the Inter-Ministerial Committee for Zoonotic Disease Control. PREDICT Malaysia then established the Zoonosis Technical Working Committee with the Department of Veterinary Services, Ministry of Health, and Department of Wildlife and National Parks (DWNP) to strengthen a national network for wildlife health and diagnostics. Traditionally, DWNP had been excluded from many discussions related to disease and wildlife surveillance. Involvement with PREDICT has helped to strengthen their capacity and role with regard to disease surveillance. For the first time since the Nipah outbreak, samples collected by DWNP have been screened at Veterinary Research Institute (VRI), and communication and collaboration between these departments has significantly improved.

In addition, PREDICT engaged DWNP and Sabah Wildlife Department in the development of a cadre of wildlife officers who are skilled in the safe capture, handling, and sampling of wildlife. Laboratory capacity was built within these departments to screen wildlife samples using broad viral family-level PCR assays. The laboratories are linked to the VRI, under the Department of Veterinary Services (DVS), which strengthened the connection between wildlife and livestock departments. Results from laboratory testing at DWNP and VRI were regularly shared with the Zoonoses Technical Working Committee, which included representatives from DWNP, DVS, and the Ministry of Health, ensuring that all three sectors were informed about novel viruses discovered at high-risk disease transmission interfaces. Training on surveillance and laboratory diagnostic techniques was provided to 130 individuals from government partners, local universities, and NGOs. This training allowed for integration of wildlife surveillance into standard operations at DWNP and SWD and contributed to the development of a One Health workforce.

In Sabah, PREDICT worked with the Sabah Wildlife Department and Danau Girang Field Centre (DGFC) to establish and develop the Wildlife Health Unit. The Wildlife Health Unit included wildlife officers from SWD Wildlife Rescue Unit and DGFC who were trained by PREDICT to be able to safely and effectively conduct wildlife disease surveillance.
Wildlife Health Unit (WHU), a new division within the Sabah Wildlife Department’s Wildlife Rescue Unit that is specifically dedicated to disease surveillance activities and managed by SWD and PREDICT-Malaysia’s Country Coordinator. The unit expands the technical expertise of wildlife rangers to include disease surveillance and has provided significant opportunities for professional development and training on topics, such as biosafety and safe wildlife handling and sampling for zoonotic agents, optimal sample handling and analysis, and molecular data analysis.

PREDICT also conducted 32 training sessions for participants from DWNP, NPHL, VRI, SWD, University Putra Malaysia, WWF Malaysia, DGFC, Sepilok Orangutan Rehabilitation Center, Borneo Sun Bear Conservation Centre, and the Institute for Tropical Biodiversity and Conservation, University Malaysia Sabah in order to build working relationships and strengthen communication among government departments, NGOs, and universities working with wildlife and/or zoonotic diseases on Peninsular Malaysia and in Sabah. Improvements in wildlife disease surveillance capacity within the DWNP resulted in a dedicated and self-sufficient team, which received funding from the Ministry of Natural Resources and Environment to support ongoing wildlife surveillance activities. This development was an important step towards connecting wildlife health experts to livestock and human health experts, thereby expanding and improving the national One Health workforce.

CAPACITY BUILDING

Surveillance Improvements
PREDICT helped to establish a consistent cold chain during surveillance activities, using portable liquid nitrogen vapor containers to maintain samples at ultra-cold temperatures in the field and during transport to the three partner laboratories (DWNP Headquarters, Wildlife Health, Genetics and Forensics Laboratory, Sabah, and VRI). Laboratory freezers were augmented with backup systems and mobile phone alarm systems in case of power or freezer failure.

Laboratory Capacity
PREDICT enhanced laboratory capacity in Sabah through establishment of the PREDICT/SWD/DGFC Wildlife Health, Genetic and Forensic Laboratory (WHGFL) in Sabah, a BSL-2 laboratory accredited and certified in accordance with CDC and NIH laboratory standards. The lab was used to conduct health checks on rescued and relocated wildlife before being released into new areas or sanctuaries, to screen samples for PREDICT and Deep Forest, and for genetic research and forensic investigations. This was SWD’s first laboratory and the first certified BSL-2 laboratory dedicated to wildlife surveillance in Malaysia.

In addition, PREDICT helped create an animal processing and sample containment room at DWNP and refurbished a dedicated BSL-2 molecular diagnostic laboratory for viral pathogen testing next to the existing wildlife forensics and genetics lab. This...
laboratory was the first-ever dedicated disease diagnostic laboratory at the Wildlife Department’s headquarters. The lab and animal processing room at DWNP served as a training center for wildlife officers and laboratory personnel.

Department of Wildlife and National Parks used findings from the PREDICT screening at this lab to strengthen their proposal for funding for a new laboratory complex and to highlight the importance of improving laboratory capacity for wildlife disease surveillance. In 2013, DWNP received substantial new funding from the Ministry of Natural Resources and Environment to create a free-standing disease diagnostics and forensics laboratory on site which will come online in 2015. This laboratory will create new jobs for molecular biologists in the wildlife conservation and health sector, which will ultimately help to expand the One Health workforce. This proposal had been rejected on five previous submissions, and its approval was in no small part due to PREDICT development of the laboratory and disease findings that allowed DWNP to justify the expense of laboratory development and show the Ministry that they had the human capacity to carry out surveillance activities.

Through a partnership with the VRI in Ipoh, PREDICT helped design and equip a BSL-2 molecular diagnostic lab within the new BSL-3 agriculture building. This lab is used to screen livestock samples as part of VRI’s routine disease surveillance but was also used for testing wildlife samples for PREDICT. VRI was a vital partner for PREDICT, providing important laboratory infrastructure and, with the Department of Wildlife and National Parks, helped PREDICT achieve the aims of the EPT program.

Each partner laboratory in Malaysia was provided reagents and primers and a universal positive control that can be used to screen for 17 viral families. PREDICT assisted with development of quality assurance and quality control standard operating procedures. PREDICT developed all standard operating procedures (SOPs) and protocols for WHGFL and helped to improve and standardize protocols and SOPs for the three laboratories it helped develop.

**Training**

PREDICT trained 130 staff from DWNP, NPHL, VRI, SWD, and various NGOs and universities in Malaysia. Training focused on field techniques for safe wildlife capture, handling, sampling, and sample transport from field to lab; laboratory techniques including molecular virology; and laboratory management and safety training. Further, PREDICT trained 21 DWNP officers from states on Peninsular Malaysia where PREDICT sampling was conducted. The DWNP established a dedicated surveillance team to conduct routine surveillance and respond to outbreaks.
PREDICT conducted four weeks of advanced laboratory training at Columbia University’s Center for Infection and Immunity, providing hands on training in high throughput sequencing (application of rapid technology to screen large amounts of genetic material) and PREDICT PCR protocols. Two members of the PREDICT Malaysia lab team: a geneticist from DWNP and the PREDICT molecular biologist travelled to New York to process wildlife samples using these techniques. High throughput sequencing was not widely available in Malaysia, but a few institutions were beginning to use this technology, so the skills will be transferrable to in-country work in the future.

**SURVEILLANCE**

In East Malaysia, PREDICT partnered with Sabah Wildlife Department (SWD) and Danau Girang Field Center. Through this partnership, intensive surveillance was carried out for the Deep Forest study (see section on Deep Forest), a coordinated study in three of the most pristine forests in the world: the Amazon Rainforest in Brazil, the Bwindi Impenetrable Forest in Uganda, and the Lower Kinabatangan River Basin in Sabah.

The Deep Forest study in Sabah was designed to provide information to PREDICT and the government of Malaysia about the effects of land conversion on the risk of zoonotic disease emergence from wildlife. The aims of the surveillance effort were to compare the abundance of key wildlife hosts (bats, rodents, and nonhuman primates) along a land-use gradient shifting from pristine forest to agricultural land to heavily populated (urbanized) environments (Figure 1) and to assess the impacts of land-use change on viral diversity and wildlife host assemblages along a deforestation gradient. Sampling was conducted at all three sites in both the dry and wet seasons, and this survey was aligned with the two parallel efforts in Uganda and Brazil.

Figure 1. Map of the lower Kinabatangan River showing Deep Forest field sites across a land-use gradient. The Deep Forest surveillance design was standardized across the land-use gradient, and included three different survey sites in each environment. Bats, rodents, and nonhuman primates were sampled at each site and screened for novel viral agents.
Surveillance was also targeted at important high-risk disease transmission interfaces between wildlife and humans, including wildlife in contact with park personnel and workers harvesting crops, wildlife in and around human dwellings or agricultural fields, and wild animals in the wildlife trade and in sanctuaries (Tables 1 and 2).

- Across Malaysia, more than 2,300 animals from priority taxa were sampled (Figure 2). In total, samples from 2,665 animals (Figures 3-4; comprised of 119 species have been screened to date for 17 viral families, including 14 viral families of public health importance (adenoviruses, astroviruses, bunyaviruses, coronaviruses, filoviruses, flaviviruses, henipaviruses, herpesviruses, influenza viruses, orthopoxviruses, paramyxoviruses, parapoxviruses, rhabdoviruses, and seadornaviruses). Samples were collected using the noninvasive, nonlethal PREDICT protocols and were tested for a panel of potential pathogens at VRI, WHGFL, DWNP, and CII. Screening at CII, DWNP, VRI, and WHGFL has identified a number of known and new viruses.
Table 1. Number of animals sampled according to targeted transmission interfaces, Sabah.

<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>14</td>
<td>181</td>
<td>254</td>
<td>22</td>
</tr>
<tr>
<td>Ecotourism and recreational activities</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>5</td>
<td>26</td>
<td>30</td>
<td>2</td>
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<tr>
<td>Wildlife being studied</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protected areas</td>
<td>71</td>
<td>64</td>
<td>450</td>
<td>15</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>13</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total:</td>
<td>129</td>
<td>271</td>
<td>735</td>
<td>44</td>
</tr>
</tbody>
</table>
Table 2. Number of animals sampled according to targeted transmission interfaces, Peninsular Malaysia

<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>197</td>
<td>18</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Ecotourism and recreational activities</td>
<td>4</td>
<td>16</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>726</td>
<td>16</td>
<td>65</td>
<td>9</td>
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<tr>
<td>Wildlife trade</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>190</td>
</tr>
<tr>
<td>Protected areas</td>
<td>0</td>
<td>77</td>
<td>59</td>
<td>7</td>
</tr>
<tr>
<td>Zoos and sanctuaries</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Total:</td>
<td>945</td>
<td>128</td>
<td>176</td>
<td>237</td>
</tr>
</tbody>
</table>

DISEASE OUTBREAK RESPONSE AND PREPAREDNESS

PREDICT trained DWNP and SWD surveillance teams on outbreak response preparedness and provided PPE and protocols to assist with outbreak response preparation. At the request of the MoH and Department of State Health in Sabah, PREDICT provided advice and assistance during a number of disease outbreaks, including sarcocystosis and leptospirosis.

In addition, Sabah Wildlife Department requested assistance from PREDICT with a pygmy elephant die-off in Sabah. PREDICT PCR protocols were used to rule out infectious disease. With coordination through USAID RDMA, the PREDICT team in Malaysia arranged for samples to be screened at AFRIMS, the Ramathibodi Poison Center in Thailand, and Queensland Biosecurity Sciences Laboratory in Australia.

REFERENCES


