excerpted from

Reducing Pandemic Risk, Promoting Global Health

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In Kathmandu, Nepal, rapid urban growth spurred by conflict and a lack of urban planning stress the vulnerability of public health infrastructure, especially in areas of intensified new growth in former croplands, forest fragments, and along urban riverbanks. Riverfront property, often the most expensive urban real estate in many cities, has been in many parts of Kathmandu converted to informal settlement and agricultural production. Along the banks of the urban rivers and their tributaries, “sukumbasi” communities (landless squatters) now comprise some of the poorest areas of dwelling in the city. These sukumbasi communities demonstrate vast ethnic and caste diversity (Tanaka 2009) and may have shifted the city’s demographic composition and ecology, as subsistence agriculture and livestock husbandry within densely populated urban areas contributes to the livelihoods of the urban poor. Both people and animals living in these settlements have limited access to basic infrastructure (e.g. clean water and treatment of sewage), and these communities fall outside of health care systems.

In addition to the settlements, Kathmandu’s complex urban ecological tapestry is rich with other opportunities for human-wildlife contact, vividly illustrated by the monkey temple context at multiple locations throughout the city. Monkey temples, common in South Asia, where primates play a role in Hindu and Buddhist culture (Fuentes 2005), are typically home to large unmanaged macaque populations. Macaque species thrive in human-altered environments and often come in close contact with humans at these temple sites (Fuentes 2005). These temple sites may account for more human-primate contact than any other context (Jones-Engel 2005), as a diverse collective of workers, visitors, pilgrims, and international travelers visit the sites and their monkey inhabitants for worship, recreation, and tourism. These sites are critical interfaces for the transmission of zoonotic diseases, and previous studies have detected viruses with zoonotic potential in resident macaque populations (Jones-Engel 2006).
Further, economic growth and rural-to-urban migration in the Kathmandu Valley has led to the rapid expansion of urban infrastructure into formerly agricultural and forested areas, degrading wildlife habitat with unknown impacts on wildlife ecology, population dynamics, and type and frequency of human-animal contact. Kathmandu Valley is home to endemic colonies of giant fruit bats (*Pteropus giganteus*), known reservoirs for multiple zoonotic viruses and long-time residents of the Valley’s forested areas, whose roosting sites are increasingly exposed to the pressures of urbanization. In part as a result of these pressures, bats at one particular site in Lazimpat, Kathmandu, roost in trees over the sidewalks of a major urban road. Bat excreta drops into pedestrian walkways, presenting a potential disease transmission risk to people and urban animal populations.

PREDICT-Nepal was initiated in 2011 (year three of PREDICT’s five year timeline), in response to feedback from the External Advisory Panel to explore the risk for disease emergence at human-animal interfaces in urban ecosystems. In Nepal, PREDICT worked to strengthen local capacity to characterize zoonotic disease risks and improve understanding of disease transmission, to lay the groundwork for disease surveillance systems for wildlife, and to assist the country in preparations for enhanced response to zoonotic disease outbreaks. Through PREDICT-Nepal’s implementing partner at the Center for Molecular Dynamics, Nepal (CMDN), research was conducted to characterize the viral diversity among taxonomic groups considered high-risk for disease emergence in Kathmandu (bats, nonhuman primates, and rodents), focusing on areas considered high-risk for human-animal contact like the *sukumbasi* informal settlements, highly-trafficked urban centers, and monkey temple complexes. PREDICT-Nepal systematically sampled urban wildlife at these interfaces and collected specimens from bats, rodents, and nonhuman primates to assess viral diversity and the potential public health significance. Findings from the project are helping to improve recommendations for disease surveillance and prevention in Nepal.

**PARTNERS**

PREDICT partners in Nepal included the Center for Molecular Dynamics Nepal (CMDN), UC Davis (UCD), and EcoHealth Alliance (EHA), working together to strengthen partnerships in-country and to establish field sites targeting areas with high levels of contact among people and wildlife, focusing on taxa that have the greatest contact with humans such as bats, rodents, and nonhuman primates.

PREDICT built upon existing CMDN relationships with in-country governmental, public, and private institutions and identified new partnerships with key agencies, ministries, and nongovernmental organizations.

Collaborators included:

- The Department of National Parks and Wildlife Conservation
- Ministry of Agriculture Development, Department of Livestock Services and Central Veterinary Laboratory
- Ministry of Health and Population, Department of Epidemiology and Disease Control
- Ministry of Health and Population, Department of Health Services National Public Health Laboratory

**PREDICT Nepal field team discusses capturing rodents with community in Kathmandu.**
• Nepal Health Research Council
• Patan Academy of Health Sciences
• The One Health Alliance of South Asia
• Tribhuvan University
• Tribhuvan University Institute of Agriculture and Animal Science
• USAID

MAJOR ACHIEVEMENTS
• Supported the organization of Nepal’s first One Health workshop in collaboration with the One Health Alliance South Asia (OHASA) Nepal Chapter to improve coordination among government, NGO, academic, and private sector stakeholders and held meetings with OHASA to share findings and discuss future collaborations leading to the formation of the One Health Association of Nepal (OHAN; see Success Stories for more information).

• Established culturally appropriate non-invasive surveillance techniques for wildlife disease surveillance (see Success Stories for more information).

• Prioritized high-risk disease transmission interfaces and locations for surveillance activities using innovative interface characterization methods through a combination of local knowledge, geographic information systems, scientific literature, feedback from ministry partners, direct observation, and unstructured interviews during field visits.

• Rapidly deployed surveillance in urban areas of Kathmandu and completed animal sampling and pathogen testing targets within an 18-month timeframe during which the PREDICT project was active in Nepal.
  – Trained 11 individuals on PREDICT animal capture, handling, and sampling protocols, and a total of 16 individuals in field surveillance techniques.
  – Selected 11 sites following intensive site characterization surveys of human-animal interfaces in the Kathmandu Valley for surveillance activities.
  – Sampled a total of 768 animals (109 bats, 411 rodents and shrews, and 248 nonhuman primates).

• Improved the infrastructure, capacity, and knowledge for viral detection in wildlife in Nepal. In less than a year, trained lab technicians at Center for Molecular Dynamics-Nepal, in close coordination with the University of California Davis laboratory team, and tested wildlife specimens for 10 viral families.

SUCCESS STORIES

One Health Association of Nepal
Nepal’s first One Health Workshop was organized in 2012 bringing government and non-government stakeholders from animal, human, and environmental health together to highlight the importance of the One Health concept as a possible means to foster cross-sector collaboration. Regional experts conducted a workshop to assess the current status of
cross communication and collaborations between various sectors and developed recommendations to initiate One Health focused efforts in Nepal. This event was co-sponsored by PREDICT and WHO/Nepal and was organized by the National Zoonoses and Food Hygiene Research Centre.

As a result of the workshop, stakeholders created the One Health Association of Nepal (OHAN), which was later registered as a non-profit organization with the Government of Nepal. Executive members of OHAN, including PREDICT-Nepal’s principal investigator, participated in the South Asia Regional Workshop for One Health held in Kathmandu, Nepal.

Following these workshops, there was greater realization from all sectors on the relevance and importance of a One Health approach in the implementation of disease surveillance, detection, prevention, and control programs. Through OHAN, Nepal developed a platform to launch initiatives connecting all health-related systems and stakeholders (animal, human, and environment) and to provide communication channels for information flow and enhanced interagency and transdisciplinary coordination.

**Culturally Appropriate Surveillance Techniques**

In Nepal, culture and religious tradition influence selection of sampling techniques for wildlife disease surveillance. Monkeys are religiously important to Hindu communities in Nepal as they represent Hanuman, a revered deity. Because invasive sampling of monkeys is restricted in Nepal, PREDICT implemented a novel non-invasive technique to collect macaque oral samples at monkey temples in Kathmandu, in partnership with UC Davis in the USA and the Mountain Gorilla Veterinary Program in Uganda.

The approach was developed and refined with laboratory colony rhesus macaques at the California National Primate Research Center, UC Davis, where optimization of sample collection, virus recovery, and virus detection was performed. In short, ropes dipped in fruit jam or banana baby food were provided to macaques to chew on and then retrieved by an attached string when discarded. Oral swab samples were also collected from the animals for comparison of virus recovery between the paired samples collected through the non-invasive and invasive methods. Following successful optimization of the technique in controlled settings and recovery of DNA and RNA viruses from the non-invasive ropes, the approach was field-tested with free-ranging arboreal and ground-dwelling non-human primates in Uganda and Nepal to evaluate both behavioral acceptance of the ropes along with recoverable sample quality in a field setting.

In Nepal, the PREDICT surveillance team worked with UC Davis veterinarians to pilot the approach among macaque communities living at three heavily trafficked temple sites, including two UNESCO world heritage sites (the Buddhist stupa Soyumbu and the Hindu temple Pashupatinath), where macaques frequently touch, scratch, and bite visitors and workers as they scavenge for food. The team evaluated multiple ways for dental rope introduction and retrieval before settling on observation and placement of a rope on the ground for macaques, then observing and following the animals until they lost interest and the swab could be safely retrieved.
and placed in a cooler for transport. Dental ropes were centrifuged by PREDICT lab technicians and then processed and tested for five viral families. Through this approach, PREDICT successfully detected RNA virus in multiple animals (Simian Foamy Virus), confirming that the non-invasive saliva collection technique using ropes is a valid sampling method for disease surveillance in difficult to capture animal populations where invasive techniques and immobilization may not be feasible (Smiley Evans et al. 2014).

CAPACITY BUILDING

Infrastructure Development

PREDICT improved wildlife surveillance capacity in Nepal through provision of animal capture and sampling supplies and cold chain equipment for sample storage. In addition, PREDICT enhanced facilities for molecular diagnostics to improve Nepal’s capacity for viral detection through the procurement of ultra-low temperature freezers and liquid nitrogen cold chain, sample storage materials, viral detection supplies including the universal control material for consensus based (genus/family level) PCR, and information management protocols through the GAINS system.

Training

Surveillance. PREDICT-Nepal trained 21 individuals in biosafety-PPE; safe animal capture and handling; laboratory safety and responsibility; rodent, primate, and bat sample collection; data recording and information management through the GAINS platform; cold chain maintenance; and safe sample shipment. In addition, intensive on-the-job training was conducted with the surveillance team on risk characterization and site assessments to refine surveillance planning and targets to best align with global and country-based objectives. During the human-animal interaction survey (see below), four field researchers and enumerators were trained on the use of a mobile application for data collection, improving data quality, and enabling feedback from the collaborative research team. Specialized training was conducted with six individuals in non-invasive saliva collection from macaques (see Success Stories above). Finally, in collaboration with partners at EcoHealth Alliance, the PREDICT Bangladesh Country Coordinator visited Nepal to conduct field-based training with eight individuals in habitat identification, roost counts, and
non-invasive sampling of urban fruit bat colonies in areas of Kathmandu where mist netting and other capture techniques were determined inappropriate or overly risky, based on the location of roosting sites in trees over heavily trafficked sidewalks, roads, and public buildings.

Pathogen Testing. PREDICT enhanced Nepal’s existing molecular diagnostic capacity by training 17 individuals in laboratory safety and six individuals in nucleic acid extraction and consensus-based (genus/family level) PCR for 10 viral families (arenaviruses, bunyaviruses, coronaviruses, filoviruses, hantaviruses, henipaviruses, influenza viruses, paramyxoviruses, retroviruses, and rhabdoviruses), equipping the project laboratory in Nepal with the capability to screen for a broad range of potential viral pandemic threats in order to support outbreak investigations for diseases of unknown origin, and networking the laboratory with global centers of excellence in advanced molecular diagnostics (e.g. deep sequencing).

SURVEILLANCE
PREDICT-Nepal focused surveillance and animal sampling efforts on urban wildlife in Kathmandu at high-risk human-animal contact interfaces filling a gap in PREDICT’s global surveillance approach (Figure 1 and Table 1). In Kathmandu, PREDICT targeted high-risk urban interfaces (areas with close human and animal contact around homes and dwellings) at seven locations for rodent and shrew surveillance in informal settlements along the Manahara, Bagmati, and Bishnumati Rivers and their tributaries. Rodents and shrews were sampled in and around human residences and dwellings and in fields and markets in urban gardens and on the urban periphery where animals damage crops and marketable produce.

Sampling was also conducted at three high-risk peri-domestic and ecotourism interfaces for nonhuman primates using non-invasive techniques. These sites are religious and tourist attractions where people are frequently touched, scratched, and bitten by macaques while feeding habituated animals. Fruit bats (*Pteropus giganteus*) were sampled non-invasively along a main thoroughfare where the bats co-roost with birds in trees lining a main urban road adjacent to a large tourist attraction, and where animal excreta fall onto heavily trafficked sidewalks. Additionally, surveillance was conducted at select sites in wet and dry seasons to assess seasonal variation in viral shedding (including repeated sampling of rodent and shrew populations at four of the unplanned settlement sites and macaques at one of the religious sites).

PREDICT-Nepal sampled a total of 768 animals (411 rodents and shrews, 248 nonhuman primates, and 109 bats; Figure 2). Specimens were prioritized for testing based on probable disease transmission pathways associated with each taxonomic group and interface, and specimens were tested for the five priority viral families for each taxonomic group in Nepal and an additional nine viral families at UC Davis. Products for sequencing were forwarded for confirmatory testing, cloning, and sequencing at UC Davis.
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>
</tr>
</thead>
<tbody>
<tr>
<td>In or near human dwellings</td>
<td>248</td>
<td>411</td>
<td>109</td>
</tr>
<tr>
<td>Total</td>
<td>248</td>
<td>411</td>
<td>109</td>
</tr>
</tbody>
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Figure 2. Number of animals sampled by taxa.
PREDICT-Nepal conducted a survey to assess human-animal contact and disease transmission risk at nine animal surveillance sites: six sites where rodents and shrews were sampled and three sites where macaques were sampled. The survey was combined with the pilot testing of an Android-platform application developed as a data collection tool to enable real-time data capture and sharing with the collaborative research team in Kathmandu and the US. Survey data were combined with test result data from animals sampled at the same sites to characterize potential disease risk and transmission pathways from animals to humans, with findings improving our understanding of the type and frequency of human-animal contact to help identify disease prevention recommendations.

PREDICT-Nepal also participated in the Local Medial Surveillance (LMS) study (see Information Management section and Schwind et al. 2014 for more information), conducting surveillance of local digital and print media sources for news of disease and health risk events not integrated into current digital disease detection systems via HealthMap, in collaboration with seven other PREDICT countries.

REFERENCES


