excerpted from
Reducing Pandemic Risk, Promoting Global Health

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Bangladesh is among the most densely populated countries on Earth, and yet, it is still rich in floral and faunal biodiversity. However, several studies indicate that 4 to 5% of faunal species (Mukul 2007) and about 10% of floral diversity (Khan et al. 2001; Dey 2006) in Bangladesh have become extinct in the last century (Mukul et al. 2008). Due to immense population pressures; over-exploitation of natural resources; habitat loss and degradation; pollution; and indiscriminate killing, hunting, and poaching of wild animals, Bangladesh’s environmental and ecological balance is under severe stress, leaving it even more vulnerable to the impacts of global climate change. Bangladesh is particularly susceptible to climate change as a result of increased frequency of natural disasters such as floods and cyclones. Losses in arable lands due to flooding pose a major threat to the food security and livelihoods of rural communities.

Bangladesh provides a unique setting for human, domestic animal, and wildlife interaction due to the co-occurrence of species-rich landscapes and high density human communities that depend heavily on intensive livestock production and also on domestic animal labor to drive agriculture. The high density of people and livestock residing in fragmented landscapes that also serve as wildlife habitat, coupled with increased sharing of scarce water and food resources, heightens the risk of zoonotic disease threats and may facilitate transmission of viruses of pandemic potential between animals and humans.

Dhaka is a city of more than 12 million people connected to other major cities around the world via a busy international airport. Many of those who live and work in Dhaka travel to their home villages during festivals and religious holidays, creating ongoing linkages between rural and urban environments and the potential for pathogen spread into a globally connected metropolis.
Bangladesh is a hotspot for emerging and re-emerging infectious diseases. Here, human and animal health are intrinsically linked, and livelihoods are highly dependent upon natural resources. Further, there is little capacity for detecting pathogens that emerge in animals prior to the occurrence of an outbreak in people. The country has recently experienced outbreaks of high and low pathogenic avian influenza (Zaman et al. 2009), H5N1 (Ahmed et al. 2010) and H1N1 pandemic influenza (Ahasan 2009), Nipah encephalitis (Luby et al. 2009), and anthrax (Chakraborty et al. 2012). As Bangladesh has one of the highest densities of human and poultry populations (World Bank 2013), there is a high risk of rapid spread in the event of an emerging virus of pandemic potential. In addition, Nipah virus continues to be a threat in at-risk rural communities in Bangladesh, with annual outbreaks with an average mortality rate of 70%. It is also a global threat, as it can be transmitted from person to person (Luby et al. 2013). These outbreaks illustrate Bangladesh’s vulnerability to large-scale epidemics of pandemic potential. It is extremely likely that the high human and animal population densities and the frequent interactions between animals and humans will continue to result in the emergence of other novel, potentially pandemic pathogens in the future.

The speed with which pathogens can emerge and spread presents serious public health, economic, and development concerns and demonstrates the need for the development of comprehensive disease detection and response capacities, particularly in hotspots like Bangladesh. Effective prevention and control of emerging diseases requires an integrated One Health approach. In Bangladesh, the PREDICT project is using a One Health approach to enhance disease surveillance systems, strengthen capacity to characterize zoonotic disease risks, improve understanding of pathogen spillover and spread, and assist the country in preparedness for response to zoonotic disease outbreaks.

PARTNERS
PREDICT partners in Bangladesh included EcoHealth Alliance (EHA), International Centre for Diarrheal Disease Research, Bangladesh (icddr,b), USAID, and Center for Infection and Immunity at Columbia University (CII).

Additional partners included:

• Bangladesh Forest Department
• Institute of Epidemiology, Disease Control and Research (IEDCR)
• Bangladesh Department of Livestock Services (DLS)
• Chittagong Veterinary and Animal Sciences University (CVASU)
• CSIRO Australian Animal Health Laboratory (AAHL)
MAJOR ACHIEVEMENTS

• Provided the first estimate of the viral diversity in a mammal species and the number of samples needed to maximize viral discovery while minimizing cost (see Success Stories for more information).

• Presented at One Health meetings and conferences across the country and conducted training workshops with FAO on One Health, thus reaching a much broader audience.

• Participated in important One Health Bangladesh workshops that brought the Ministry of Health, the Department of Livestock Services, and the Forest Department together for the first time. The Director General of each ministry signed a document indicating that they will follow One Health principles within their respective ministries.

• Trained 29 biologists, students, and veterinarians, including the first veterinarian hired by the Bangladesh Forest Department, in collaboration with PREDICT’s in-country partner, International Centre for Diarrheal Disease Research, Bangladesh.

• Collected samples from 5,796 animals during the project. A total of 2,435 bat samples were tested by PCR for 11 viral families, 840 rodent and shrew samples were analyzed by PCR for 10 viral families, and 466 nonhuman primate samples were analyzed by PCR for 34 viral families and genera.

• Partnered with icddr,b, which has a molecular biology laboratory with equipment and technical expertise to screen wildlife samples. Introduced the PREDICT PCR protocols, primers, and positive controls to augment lab surveillance capabilities.

• Supported the government and icddr,b’s ongoing response efforts during annual outbreaks of Nipah virus.

• Supported IEDCR laboratories by providing PREDICT PCR protocols to be used for human surveillance activities.

SUCCESS STORIES

PREDICT-Bangladesh provided the first ever estimate of total diversity of known and novel viruses in a mammalian species (Anthony et al. 2013). The team used a novel strategy that integrated concepts from viral discovery, phylogeny, and ecological analyses to estimate the approximate viral diversity in the Indian flying fox (Pteropus giganteus), a native frugivorous bat.

Training a veterinarian from the Bangladesh Forest Department in sampling wild birds for avian influenza.
The PREDICT team repeatedly sampled these bats and utilized archived samples to conduct deep sequencing for viral discovery on over 1,800 individual bats. This approach resulted in the discovery of 55 viruses belonging to nine viral families known to include zoonoses. Using ecological analyses developed to predict biodiversity of species in ecosystems, the PREDICT team estimated that the total diversity in these viral families in this bat species is 58, suggesting that the surveillance strategy was effective in identifying the majority of novel viruses that may be shed by a given species. From this effort, the PREDICT team estimated that there are approximately 320,000 total viruses in mammals globally, and that discovery of 80% of the unknown global diversity of viruses in mammals would cost approximately USD $1.4 billion – a fraction of the estimated $30 billion in damages inflicted by just one outbreak – the SARS virus pandemic (Anthony et al. 2013).

CAPACITY BUILDING
PREDICT-Bangladesh used a collaborative One Health approach to build capacity for detection and characterization of novel wildlife viruses that potentially pose a significant public health threat.

Training
PREDICT trained over 29 individuals in topics including zoonotic diseases, sampling methodology, human and animal safety during wildlife capture, personal protective equipment (PPE), and laboratory methodology and safety. Trainees included the Bangladesh Forest Department’s expanding veterinary staff in collaboration with icddr,b. The veterinarians were provided training in basic epidemiology, disease surveillance in wildlife, and outbreak investigation. The PREDICT Bangladesh team also worked with the PREDICT Nepal team to assist with bat sampling and train local team members to safely collect and handle bat samples.

We also conducted trainings at local government and FAO-sponsored One Health meetings, and through these meetings, PREDICT shared techniques and technologies and strengthened collaborations among the Bangladesh Forest Department, Department of Livestock Services, IEDCR, and Chittagong Veterinary and Animal Sciences University.

Laboratory Capacity
To enhance laboratory capacity in Bangladesh, PREDICT PCR protocols (which target viral families and allow for the detection of unknown viruses) and positive controls were used in wildlife sample testing at the icddr,b laboratory and will be provided to IEDCR where they will be used for human disease surveillance.

Building a One Health Network
Supporting a One Health framework in Bangladesh is an important focus for PREDICT personnel who are members of One Health, Bangladesh (OHB) and the One Health Alliance of South Asia (OHASA), a regional One Health network. The PREDICT team participated regularly in OHB meetings, workshops, and One Health conferences. The team was also
involved in developing a strategic framework for a One Health approach to infectious diseases in Bangladesh with the Ministry of Health, the Department of Livestock Services, the Forest Department, USAID, and iccdr,b. These activities reflect the commitment that these government agencies made to incorporate One Health into their mandates.

SURVEILLANCE
The surveillance strategy in Bangladesh began with targeted sampling of mammals and wild birds that had a high degree of contact with people in rural and urban settings but quickly focused down on three key taxonomic groups that were highly associated with people and that were most likely to harbor zoonotic viruses: bats, rodents, and nonhuman primates.

Several key high-risk disease transmission interfaces were targeted; however, due to the population density of Bangladesh, the team focused on the peri-domestic interface by sampling animals in and around dwellings (77% of sampled animals). Other high-risk interfaces were targeted by sampling animals that were raiding markets; animals that have contact with researchers, park personnel and tourists; and animals that have little to no contact with people but live in locations where indigenous people hunt (Figure1 and Table 1).

Figure 1. Sites where PREDICT conducted virus surveillance in wildlife taxa at high-risk disease transmission interfaces between wildlife and humans.
A total of 5,796 animals (4,110 bats, 732 rodents and shrews, 543 nonhuman primates, 332 birds, 50 ungulates, eight carnivores, and 21 animals from other taxa; Figure 2) were sampled. A large number of shrews were sampled along with the rodents because, in Bangladesh, shrews are more common in human dwellings than rodents.

**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>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Ecotourism and recreational activities</td>
<td>4</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>In or near human dwellings</td>
<td>539</td>
<td>371</td>
<td>3905</td>
<td>28</td>
</tr>
<tr>
<td>Wildlife raiding markets</td>
<td>0</td>
<td>84</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>Pristine habitat</td>
<td>0</td>
<td>70</td>
<td>114</td>
<td>383</td>
</tr>
<tr>
<td>Other high-risk interfaces</td>
<td>0</td>
<td>188</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>543</strong></td>
<td><strong>732</strong></td>
<td><strong>4110</strong></td>
<td><strong>411</strong></td>
</tr>
</tbody>
</table>

**DISEASE OUTBREAK RESPONSE AND PREPAREDNESS**

Bangladesh has experienced repeated outbreaks of zoonotic diseases including Nipah virus, avian influenza, and cutaneous anthrax. In response, the Bangladesh government has developed a sophisticated One Health approach to zoonotic disease outbreak response. PREDICT staff supported the Government of Bangladesh and local implementing partners at the Ministry of Health (IEDCR) and icddr,b by conducting wildlife sampling and training of Forestry Office staff in the safe collection of wildlife samples during Nipah virus outbreak investigations.

**VIRUS DISCOVERY AND CHARACTERIZATION**

Using data generated from surveillance activities in Bangladesh, PREDICT provided proof-of-concept for a strategy to estimate viral richness within one mammalian species. PREDICT viral discovery protocols were applied to specimens from repeatedly sampled *Pteropus giganteus* fruit bats to saturate the discovery of new viruses (Anthony et al. 2013). This species was selected...
because of its role as the reservoir for Nipah virus, which is transmitted through contamination of date palm sap with saliva or urine when bats feed on the sap overnight. Date palm sap is an important interface for bat-to-human spillover of Nipah virus, and may also represent a potential route of transmission for some of the newly discovered viruses as well. Among samples tested from 1,800 bats, 50 novel and 5 known viruses (based on clade analysis) from nine viral families (coronaviruses, paramyxoviruses, astroviruses, influenza A viruses, adenoviruses, polyomaviruses, bocaviruses, and herpesviruses) were discovered.

Analyses commonly used for estimating biodiversity in ecological studies revealed that there are a minimum of 320,000 mammalian viruses awaiting discovery within these nine families assuming all species harbor a similar number of viruses with minimal turnover between host species. The team also predicted that 80% of the viruses in a species could be estimated through testing of only 500 samples from individual animals (Figure 3).

Figure 3. Viral discovery curve. A subset of 1,092 samples were tested for all nine viral families and 44 viruses (out of a total of 55) are represented. The 11 viruses not considered were PgHV-2, -5,-6, and -9; PgAdV-1 and -10, PgAstV-4, -5, -6, and -8, and PgBoV-1. Black line, the rarefaction curve; redline, collector curve showing accumulation of novel viruses over samples tested; blue line, Chao2 estimator at every sample point, with arrow indicating 95% confidence intervals; gray lines, ICE and Jackknife estimators at every sample point; dashed vertical lines, required sampling effort to discover an arbitrary proportion of the total diversity (including 100%); horizontal line, total estimated diversity, 58 viruses, and effort required to discover 100% of the estimated diversity, 7,079 samples. From Anthony et al. (2013).
REFERENCES


World Bank. 2013. Implementation completion and results report (IDA-43400 TF-90662) on a credit in the amount of SDR 10.5 million (US$16.0 million equivalent) to the People’s Republic of Bangladesh for an avian influenza preparedness and response project under the global program for avian influenza and human pandemic preparedness and response. Washington, DC. Available at: http://www-wds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2013/07/04/000442464_20130704100805/Rendered/INDEX/ICR21770ICR0Av0Box0377341B00PUBLIC0.txt.