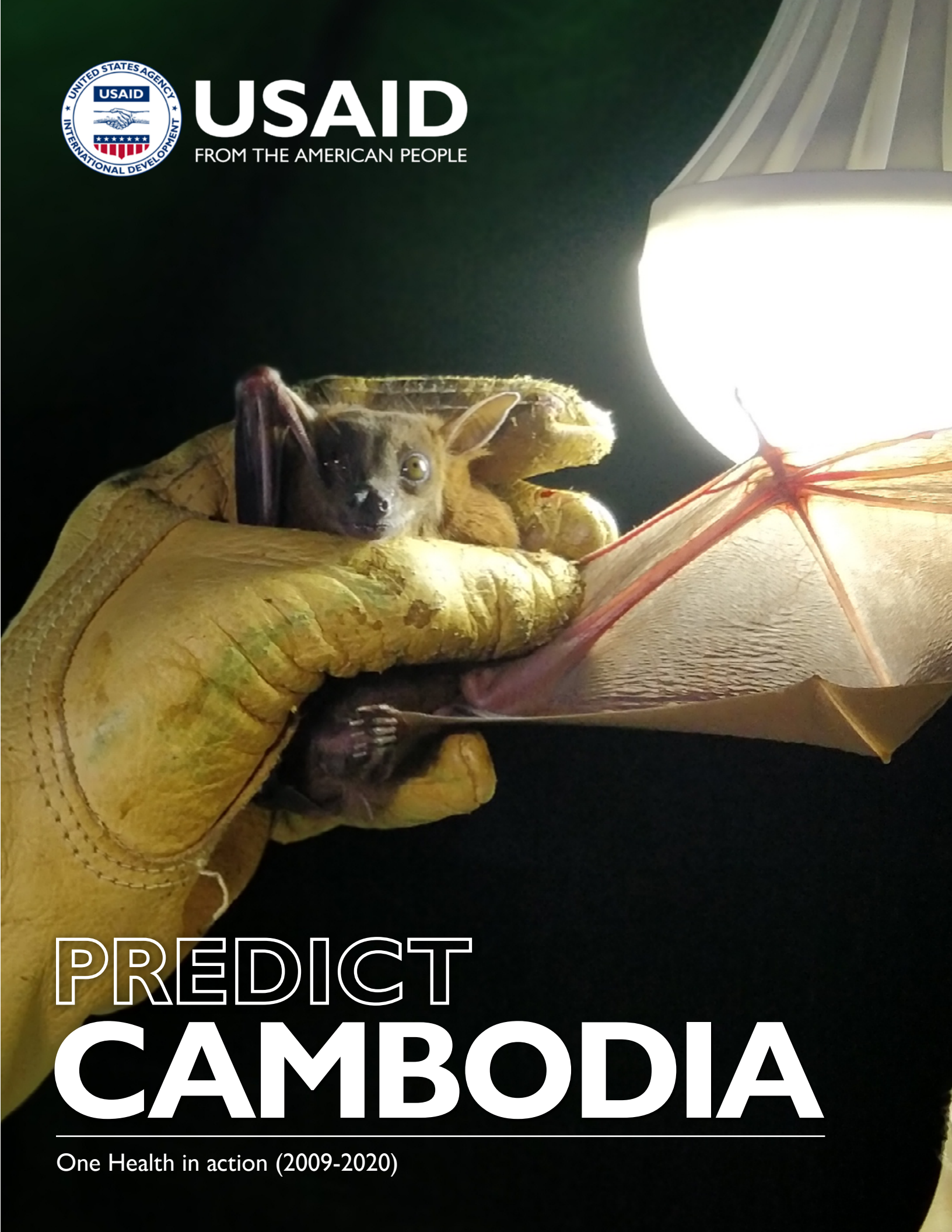




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PREDICT CAMBODIA

One Health in action (2009-2020)



Improving the capacity to detect known and emerging viruses in Cambodia through a One Health approach that includes training of in-service government partners from the human health, animal health, and forestry sectors, as well as students, to perform field surveillance, laboratory testing, data interpretation, and analyses.

CAMBODIA

During the first phase of the PREDICT project (PREDICT-1; 2009-2014) 3,887 wild animals were safely and humanely sampled including non-human primates, rodents, and bats. We targeted diverse wildlife-human interfaces for surveillance including wildlife hunting and trade; crop raiding by wild animals; wildlife rescue centers; markets and restaurants selling wildlife; bat guano farms; religious and ecotourism sites; and rodents traded to Viet Nam for food to get a better understanding of where risk for spillover of viruses from wild animals to humans was occurring. Samples were safely tested via polymerase chain reaction (PCR) for 16 viral families at Institut Pasteur du Cambodge (IPC), resulting in the detection of 18 known and 29 novel viruses.

During the second phase of the PREDICT project (PREDICT-2; 2015-2019), surveillance was focused on two high-risk interfaces based on knowledge gained during PREDICT-1. The PREDICT/Cambodia team targeted intensifying animal production in the bat guano trade in Kampong Cham Province, and the market value chain of the cross-border rodent trade

to Viet Nam in Kandal province. We performed concurrent longitudinal surveillance of people, their animals, and wildlife at these communities and surveyed people who were sampled to better understand risk for virus spillover and to inform on options for behavioral change to decrease risk. We also performed syndromic surveillance of febrile patients who enrolled at any of the three clinics that serve these two high-risk communities.

Viral family testing was performed at IPC for seven viral families/genera with concurrent training and transferring of PREDICT project protocols to the national animal and human laboratories. During PREDICT-2, samples were safely collected from 1,803 people, 2,715 domestic animals, and 5,890 wild animals, including 875 rodents and 5,006 bats. This work resulted in the detection 19 known and 17 novel PREDICT project-discovered viruses. Of these, 2 known and 4 novel PREDICT project viruses were previously detected in PREDICT-1. In total, the PREDICT team in Cambodia detected 35 known viruses and 42 novel viruses throughout the ten year period, for a total of 77 unique viruses.

Viruses with zoonotic potential and those that can affect the productivity and health of livestock, were identified in animals. Full genome sequencing was performed to further characterize seven of these viruses. Our testing led to the detection of viruses not routinely tested for in people presenting with Influenza Like Illness (ILI), Severe Acute Respiratory Illness (SARI), Fever of Unknown Origin (FUO), hemorrhagic fever and/ or encephalitis in Cambodia, thus expanding the understanding of viruses circulating in people.

The second phase of the PREDICT project also supported One Health initiatives for effective collaboration across disciplines and government sectors, including engagement with the Cambodian Zoonotic Technical Working Group and veterinary and human health university faculties. Through analysis of project data and findings, the PREDICT team was able to identify risks and educate communities and health professionals on behavior change and intervention strategies designed to protect people and wildlife from disease threats.

IMPLEMENTING PARTNERS

- University of California, Davis: PREDICT project lead and global implementing partner
- Institut Pasteur du Cambodge: PREDICT-2 lead and implementing partner in Cambodia
- Ministry of Health: Department of Communicable Disease Control (CDC)
- Ministry of Agriculture and Forestry: General Directorate of Animal Health and Production (GDAH)
- Ministry of Agriculture and Forestry: National Animal Health and Production Research Institute (NAHPRI)
- Ministry of Agriculture and Forestry: Forestry Administrations' Department of Wildlife and Biodiversity (DWB)
- Food and Agricultural Organization (FAO)
- Wildlife Conservation Society
- USAID

OTHER KEY LOCAL PARTNERS

- National Institute of Public Health (NIPH)
- Royal University of Agriculture (RUA)
- University of Health Sciences (UHS)
- Royal University of Phnom Penh (RUPP)
- Kaoh Thum Referral Hospital
- Kunthabopha Hospital
- Two hospitals in Prey Chhor District
- Kang Meas District veterinarian & veterinarian health workers
- Kaoh Thum District veterinarian & veterinarian health workers
- Chiefs of local villages in Kaoh Thum and Kang Meas Districts



300

DEVELOPED the One Health Workforce by training 300 people in Cambodia.

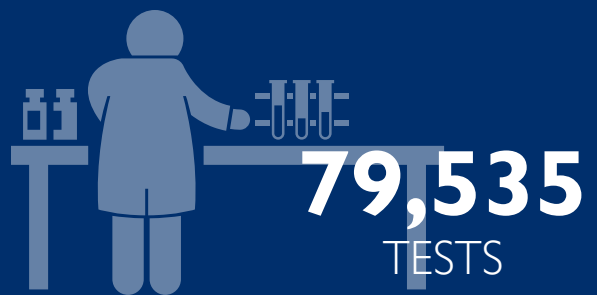


>14.1K

OPERATIONALIZED One Health surveillance and sampled over 5.7K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

· Institut Pasteur du Cambodge



79,535
TESTS



42

PREDICT

35

KNOWN

DETECTED 77 unique viruses in both animal and human populations.



VEASNA DUONG

Senior Research Associate
Institut Pasteur du Cambodge

“I believe PREDICT has brought many benefits to Cambodia, especially through the laboratory protocols to identify known and newly emerging viruses, through protocols for handling and sampling animals in the field, and in the training of government and students in those protocols. Also, PREDICT has benefitted communities we work with by bringing them knowledge of how they can lower their risk of contracting zoonotic disease both from the wild animals they live closely with and from other animals, too.”



VIBOL HUL

Field Coordinator & Senior
Laboratory Technician
Institut Pasteur du Cambodge

“In the 10 years I’ve worked on PREDICT, I’ve learned how to address challenges in the laboratory and also in our field work related to animal handling, including wildlife and domestic animals, cold chain, biosafety and biosecurity. Using PREDICT’s surveillance results, I also have the opportunity to share my knowledge of zoonotic diseases with the community, students and government staff. Those experiences have inspired me to pursue a Ph.D. in France on Arenavirus in rodents later this year.”

ACHIEVEMENTS

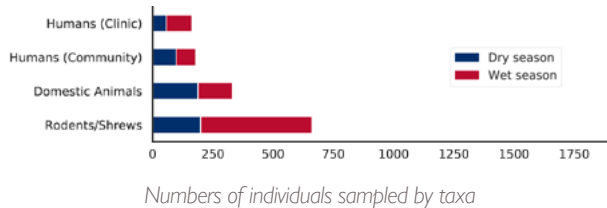
- PREDICT-2 performed the first ever concurrent sampling of wildlife, domestic animals and people in Cambodia, with excellent collaboration from the human, animal and forestry sectors of the government. These multi-disciplinary teams were trained in One Health implementation from the field to the laboratory.
- Thirty-three students from the schools of veterinary medicine (8), medicine (2) and epidemiology (23), 13 of whom were women, were trained in PREDICT project laboratory and field sampling protocols. Two of these students have since been hired as permanent staff at Institut Pasteur du Cambodge, and one has received a master’s program scholarship for the One Health-focused InterRisk program at Kasetsart University in Thailand (www.onehealthsea.org/interrisk). These personal successes reflect the significance and workforce impacts of the project in Cambodia.
- PREDICT project PCR protocols have been adopted for use in outbreaks by the Cambodian government. Two examples include the detection of Influenza A (subtyped as H5N1) in a hairy-nosed otter at the Phnom Tamao Zoo, and the detection of Ursid gammaherpes virus after a disease outbreak in bears, also at the zoo.
- The PREDICT team in Cambodia was able to detect SARS-CoV-2, the virus causing the current COVID-19 pandemic, early-on in the outbreak by using PREDICT project test protocols already in use before the SARS-CoV-2 genome was available and before the development of SARS-CoV-2 specific real-time PCR assays.

ONE HEALTH SURVEILLANCE

The PREDICT project's One Health surveillance approach was designed to strengthen capacity for detection of emerging viral threats and to improve our understanding of risk of zoonotic diseases in communities with close and frequent animal contact. The PREDICT team performed the first ever concurrent sampling of wildlife (bats and rodents), domestic animals and people in Cambodia, targeting high risk populations for virus spillover, amplification, and spread. The team sampled at a bat guano farming community and at a international rodent trade hub. Longitudinal surveillance was conducted over a four-year period during both the rainy and dry

seasons, with multi-disciplinary teams from the human and animal health and forestry sectors receiving trainings in One Health approach implementation from the field to the laboratory. Species identification of bats and rodents was confirmed using DNA barcoding, a molecular-based laboratory assay. Surveys of humans were performed at the time as of sampling, to better understand behaviors that may increase risk of viral spillover from wild animals to people. The team also conducted syndromic surveillance of patients presenting to local clinics serving the communities at our sites.

KAOH THUM DISTRICT RODENT TRADE HUB

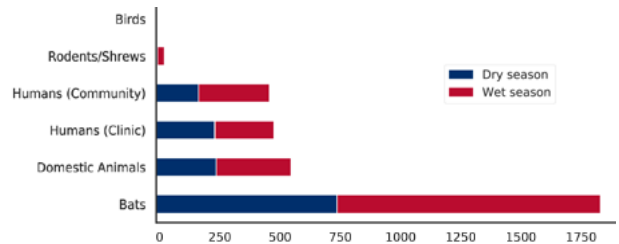


Numbers of individuals sampled by taxa

A potential high-risk market value chain for disease spillover and spread is the cross-border rodent trade from Cambodia to Viet Nam, and was therefore selected as a surveillance site. Rodents are trapped across Cambodia and brought, live and in overcrowded conditions, by middlemen to the rodent trade hub site in Kaoh Thum District, Kandal Province where they are sorted before being transported across the border to Viet Nam to be sold for food. Thousands of rodents a week are traded in the peak season. Sampling targeted the captured rodents, domestic animals at and around the trade hub, and people (trappers, drivers, middle men, children assisting with handling). We also conducted behavioral surveys to explore the practices and behaviors of these individuals that have the potential to facilitate spillover and spread of zoonotic viruses.

Syndromic surveillance was also conducted at a clinic within Kaoh Thum District that serves the local community.

KANG MEAS DISTRICT BAT GUANO FARMING COMMUNITY



Numbers of individuals sampled by taxa

Our team conducted sampling in rural communities within Kang Meas District, Kampong Cham Province, where construction of artificial bat roosts for the collection of bat guano is common. Roosts are constructed in close proximity to peoples' homes, their children, livestock, and the crops they raise to support their livelihoods. Sampling targeted wildlife (bats and rodents), livestock (coordinated with FAO), and people (farmers, their family, guano middlemen). We also conducted behavioral surveys to explore the practices and behaviors of these individuals that have the potential to facilitate spillover and spread of zoonotic viruses.

In addition to the community surveillance, syndromic surveillance was performed at two clinics serving the local communities, located in Prey Chhor District.



VIRUS DETECTION

The testing strategy in Cambodia for virus detection included screening animal and human samples using broadly reactive consensus PCR (cPCR) for seven viral families/ genera, including corona, filo, flavi, hanta, influenza, paramyxo and rhabdo viruses.

VIRUS FINDINGS IN PEOPLE

Of the 650 patients sampled at hospitals, 23 were positive for at least one virus. Fifteen people were positive for Influenza A, 4 for Influenza B, 1 for Human coronavirus (HKU-1), and 3 for Mumps virus. Except for Influenza A, none of these viruses are routinely tested for or would have been detected through the routine national surveillance system.

VIRUS FINDINGS IN ANIMALS

Seventeen viruses were detected in bats of which 16 were novel (4 coronaviruses, 4 paramyxoviruses and 8 rhabdoviruses) and 1 was a known coronavirus. Six

known viruses were detected in rodents including 2 hantaviruses and 4 coronaviruses. Of importance was the detection of the hantavirus, Seoul virus, in rodents within both districts, as this known zoonotic pathogen can cause respiratory and hemorrhagic illness in people. Eight viruses were detected in domestic animals including 1 novel coronavirus and 7 known viruses (4 coronaviruses, 1 influenza virus, 1 paramyxovirus, and 1 flavivirus). The detection of Infectious bronchitis virus, Influenza A, Newcastle disease virus, and Tembusu virus in domestic animals were noteworthy, as these are viruses are known to affect the health and productivity of these animals.

VIRUS TABLE

VIRAL FAMILY	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Human coronavirus HKU1	Human	Hospital 1 (Prey Chhor District)	1	0	1
	PREDICT_CoV-24	Greater Short-Nosed Fruit Bat	Kang Meas District	2	1	1
	PREDICT_CoV-25	Lesser Asian House Bat	Kang Meas District	1	1	0
	PREDICT_CoV-35	Lesser Asian House Bat	Kang Meas District	4	2	2
	PREDICT_CoV-56	Greater Short-Nosed Fruit Bat	Kang Meas District	2	1	1
	Bat coronavirus 512/2005	Lesser Asian House Bat	Kang Meas District	83	43	40
	Longquan Aa mouse coronavirus	Greater Bandicoot Rat, Ricefield Rat, Lesser Ricefield Rat, Unidentified Rattus Rat	Kaoh Thum District	70	36	34
	Murine coronavirus	Greater Bandicoot Rat, Ricefield Rat, Lesser Ricefield Rat, Ricefield	Kaoh Thum District	190	113	77
	Rodent coronavirus	Ricefield Rat, Ricefield Mouse, Ricefield Rat	Kaoh Thum District	6	3	3
	PREDICT_CoV-100	Domestic Chicken	Kaoh Thum District, Kang Meas District	41	20	21
	Betacoronavirus 1 (Bovine CoV)	Domestic Cow	Kang Meas District	1	0	1
	Betacoronavirus 1 (Canine strain)	Domestic Dog	Kaoh Thum District	1	1	0
	Duck coronavirus	Domestic Chicken	Kaoh Thum District	8	8	0
	Infectious bronchitis virus	Domestic Chicken, Domestic Duck	Kaoh Thum District, Kang Meas District	92	72	20
	Paramyxovirus	Human parainfluenzavirus 1	Human	Kunthabopha Hospital	2	0
Human parainfluenzavirus 3		Human	Kunthabopha Hospital	1	0	1
Mumps virus		Human	Hospital 1 (Prey Chhor District)	3	1	2
PREDICT_PMV-13		Lesser Asian House Bat, Whiskered Myotis	Kang Meas District	92	66	26
PREDICT_PMV-63		Lesser Asian House Bat	Kang Meas District	6	4	2
PREDICT_PMV-66		Lesser Asian House Bat	Kang Meas District	31	23	8
PREDICT_PMV-67		Lesser Asian House Bat	Kang Meas District	19	17	2
Newcastle disease virus		Domestic Chicken	Kaoh Thum District, Kang Meas District	3	2	1
Influenza virus	Influenza A	Human, Domestic Chicken, Domestic Duck	Kaoh Thum District, Kang Meas District, Hospitals 1 & 2 (Prey Chhor District)	25	15	10
	Influenza B	Human	Hospitals 1 & 2 (Prey Chhor District)	5	4	1
Flavivirus	Tembusu virus	Domestic Chicken	Kang Meas District	4	2	2
Rhabdovirus	PREDICT_RbdV-21	Lesser Asian House Bat	Kang Meas District	14	8	6
	PREDICT_RbdV-27	Lesser Asian House Bat	Kang Meas District	20	3	17
	PREDICT_RbdV-28	Lesser Asian House Bat	Kang Meas District	7	6	1
	PREDICT_RbdV-31	Lesser Asian House Bat	Kang Meas District	2	2	0
	PREDICT_RbdV-32	Lesser Asian House Bat	Kang Meas District	1	1	0
	PREDICT_RbdV-33	Lesser Asian House Bat	Kang Meas District	1	1	0
	PREDICT_RbdV-34	Lesser Asian House Bat	Kang Meas District	3	3	0
	PREDICT_RbdV-35	Lesser Asian House Bat	Kang Meas District	1	0	1
Hantavirus	Seoul virus	Ricefield Rat	Kaoh Thum District	2	1	1
	Thottapalayam virus	Asian House Shrew	Kang Meas District	2	2	0
Total				746	462	284

EPIDEMIOLOGIC & BEHAVIORAL RISK

Our PREDICT team partnered with local communities to learn more about people's perceptions of risk for exposure to zoonotic diseases and how the types of activities and contact they engage in might influence the risk of disease exposure. Surveys included questions on human demographics, livelihood activities, animal contact, and food safety and sanitation practices. A total of 893 individuals were enrolled in the community-based surveillance - 565

from the bat guano farming community in Kang Meas District and 328 from the rodent trade site in Kaoh Thum District. An additional 836 individuals were enrolled through the syndromic surveillance conducted at the three clinics serving the two communities. Participants were enrolled throughout the year across the rainy and dry seasons, with surveys and sampling occurring concurrently after obtaining informed consent.

	CLINIC SURVEY PARTICIPANTS			COMMUNITY SURVEY PARTICIPANTS	
SITES	HOSPITAL Kaoh Thum District (n=294)	HOSPITAL 1 Prey Chhor District* (n=225)	HOSPITAL 2 Prey Chhor District* (n=317)	Kaoh Thum District (n=328)	Kang Meas District (n=565)
GENDER					
FEMALE	147 (50%)	102 (45.3%)	186 (48.6%)	203 (61.9%)	346 (61.2%)
MALE	147 (50%)	123 (54.7%)	131 (51.4%)	125 (38.1%)	219 (38.8%)

*Hospitals in Prey Chhor District served the community members of Kang Meas District

Data from the questionnaires identified behaviors and household characteristics that could increase risk of viral spillover from animals to humans in both villages.

Within the Kang Meas District community, 33% of people obtain their drinking water from an uncovered source such as a rainwater cistern, well or pond. At the more developed Kaoh Thum District site, this number was lower at 13%. Such open water sources are not protected from contamination by bat or bird feces or urine from animals living above them, which could contain infectious material. Furthermore, 59% percent of people surveyed in Kang Meas District and 18% in Kaoh Thum District reported sharing their drinking water source with animals. Risky behaviors associated with food consumption included eating food contaminated by animal feces (24% of people in Kaoh Thum District and 27% in Kang Meas District).

People in both communities have livelihoods that lead to a higher than usual contact with bats or rodents, but

they reported (and were observed) using limited personal protection while handling bats or rodents, and their feces and urine. A quarter of respondents working in the rodent trade and more than half working on bat guano farms reported wearing no protective clothing. Of those that described wearing some protective gear, a simple hat or cloth gloves were the most common items worn. No one reported using adequate personal protective equipment to reduce risk for disease exposure.

The questionnaire captured participants' lack of awareness of potential disease transmission routes from animals to humans as 84% of respondents in Kaoh Thum District and 72% in Kang Meas District thought there was no health risk or didn't know of any risk from animal bites (Figure 4). Thus, community members did not seek adequate medical treatment when bites or injuries occurred while handling animals. Most (56% in Kaoh Thum District and 50% in Kang Meas District) did nothing following a bite, including not cleaning a wound with water.



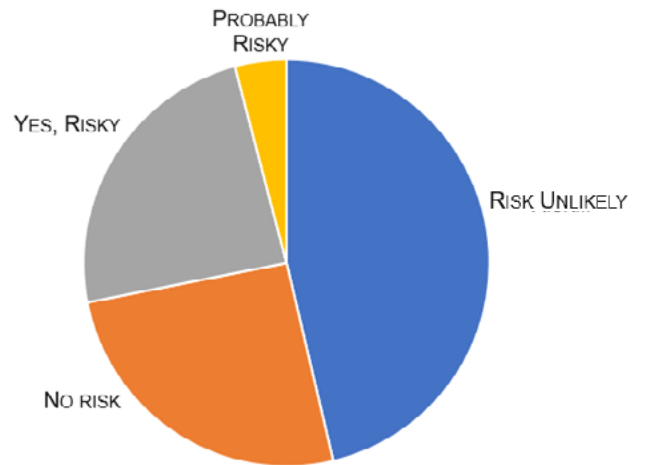
COMMUNITY ENGAGEMENT & RISK COMMUNICATION

Throughout the project, we held meetings with our partnering communities and with the clinics that participated in syndromic surveillance to discuss how and why the PREDICT project was implemented in Cambodia, shared testing results from human, domestic animal, and wildlife samples, and informed on risky behaviors identified through questionnaires. Some of these behaviors included sharing of drinking water sources and food with animals, eating food contaminated with animal feces, lack of use of Personal Protective Equipment (PPE) when handling animals and their guano, and not understanding the risks from infection following animal bites. We discussed how community members could reduce the risk of exposure to diseases from animals and used the *Living Safely With Bats* book (translated into Khmer), a resource developed by the PREDICT project, as a tool to discuss how to reduce risk.

The communities greatly appreciated these meetings and the opportunity to learn about our testing and questionnaire results. Individuals were very interested in the virus findings, particularly about the viruses that pose a risk to their health or that of their animals. Copies of *Living Safely with Bats* were left with the chiefs of the villages, the local veterinarians, bat farmers, and in libraries at the pagodas and elementary schools so that information could be more widely shared throughout the communities.

Doctors and nurses from the clinics that serve the communities also valued discussion of the viral findings and questionnaires, such as the detection of the zoonotic Seoul virus in rodents, and of the contact patients reported with animals, which could lead to an increased risk of exposure to disease. The *Living Safely With Bats* book was seen as a valuable tool to educate on disease prevention, and doctors requested an electronic version of the book to be produced for use in the waiting rooms at their clinics.

KANG MEAS DISTRICT



KAOH THUM DISTRICT

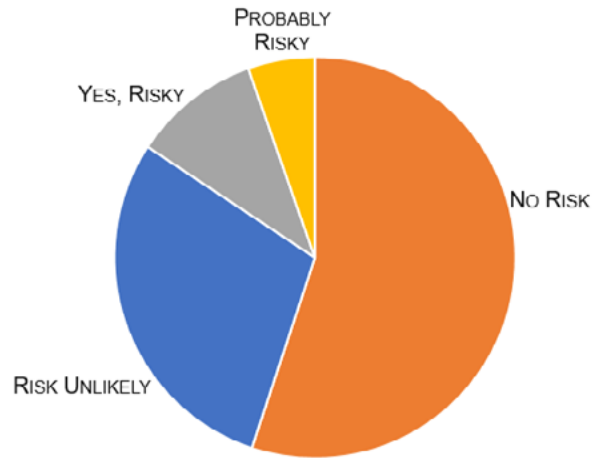


Figure 4: Perception of Whether Animal Bites Pose a Risk For Disease Transmission in Kang Meas District (top) and Kaoh Thum District (bottom)



STRENGTHENING CAPACITY

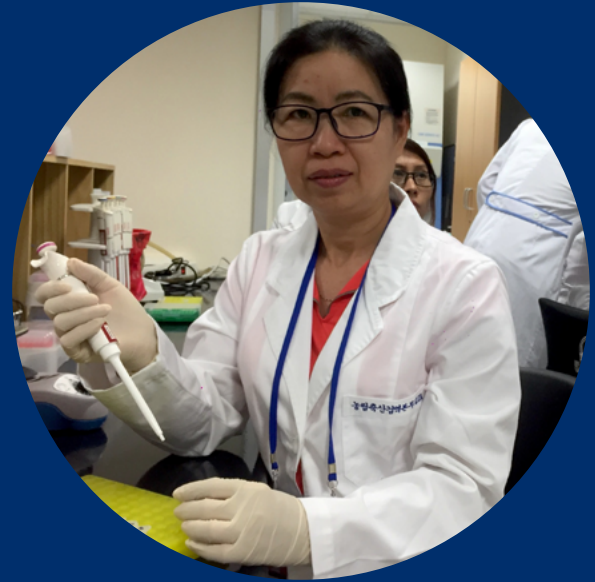
One Health capacity building was a key focus of the PREDICT project and we conducted trainings in the classroom, field, and laboratory for students and in-service government staff from multiple sectors across the One Health spectrum. Trainings developed skills in biosafety and PPE use, safe capture and sampling of wildlife and domestic animals, specimen handling and maintenance of a cold chain, virus detection using consensus PCR, and disease outbreak response.

Twenty-five Cambodian government staff participated in PREDICT project trainings, many of whom also participated in concurrent sampling. Training from the field to the laboratory was provided to government staff from the Cambodian CDC, the Forestry Administration and the National Animal Health and Production Institute (NAHPRI). Repeat training for government counterparts on safe animal capture and sampling was conducted as part of the project's close-out, to facilitate sustainability of activities beyond the end of the project. Staff from the government's animal and human health laboratories (NAHPRI and the National Institute for Public Health (NIPH)), were trained at the Institut Pasteur du Cambodge to use PREDICT protocols for broadly reactive consensus PCR as a tool to detect both known and unknown viruses.

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ONE HEALTH WORKFORCE: THE NEXT GENERATION



“From PREDICT I have gained knowledge that the wildlife can be a reservoir for a lot of zoonotic viruses and have gained a lot of experiences on methods such as how to collect specimens from bats and rodents in the field and also testing for viruses in the laboratory. PREDICT has been valuable in Cambodia for the good communication and collaboration between IPC and NAHPRI, with IPC training our staff in PREDICT laboratory techniques. The project has identified some viruses in domestic animals and wildlife for the first time in Cambodia and has provided great awareness for early prevention of disease between animal and human. Finally, PREDICT gave a lot of advances for scientists here in the One Health approach.”

REN THEARY is a veterinarian working in the Cambodian government's National Animal Health and Production Institute since 2000. Theary participated in the PREDICT project's concurrent sampling events and sample testing in Cambodia.

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