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PREDICT THAILAND

One Health in action (2009-2020)



10 years of PREDICT activities in Thailand has developed leaders in the One Health approach to pandemic preparedness, outbreak response, and viral discovery.

THAILAND



Thailand is a biologically diverse country where ecological changes caused by land conversion and agricultural production are bringing humans into increased contact with wildlife and the pathogens they carry. A diversity of novel wildlife viruses with potential economic and public health importance are circulating in Thailand while the threat posed by pathogens like Nipah virus, coronaviruses, and influenza virus highlights the urgent need to support ongoing research, surveillance, and capacity building efforts. Local community interactions with wildlife in rural settings (e.g. with macaques or flying foxes in temples, or with bats while harvesting guano for fertilizer) represent additional interfaces for zoonotic disease spillover. Bangkok and other areas of Thailand are hubs for the wildlife trade and regional centers of travel and tourism. Thailand is centrally connected via porous borders with Laos, Cambodia, Malaysia, and Myanmar. The high volume of cross-border movement of people, their animals, and trafficked wildlife poses an added risk for transboundary pathogen introduction and spread, including infected travelers bringing in MERS-CoV, Zika virus, Ebola virus, and SARS-CoV-2.

PREDICT in Thailand is led by the team at the World Health Organization (WHO) Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University and the Thai Red Cross Emerging Infectious Diseases Health Science Centre, in collaboration with the Faculty

of Forestry at Kasetsart University, the Department of National Parks, Wildlife and Plant Conservation, and additional government partners in human and animal health. The project leverages these groups' expertise discovering novel viruses in humans and animals, and has increased these capabilities, especially for surveillance of specific pathogens. Capacity building is essential to the sustainability of the program. PREDICT project activities helped build the skills, systems, infrastructure, and human resources for emerging infectious disease preparedness within the teams and institutions involved in the project, and on a larger scale as these skills and practices have been adopted by additional government partners.

Ten years of PREDICT project activities helped strengthen an early warning 'One Health' system for emerging infectious disease (EID) preparedness capacity in Thailand, as implementing partners gained expertise in safe and humane wildlife sampling, human sample collection for unknown disease detection, behavioral risk research, laboratory testing and cold chain, and disease modelling and data analysis. In addition, 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. PREDICT project PCR protocols have been widely implemented to identify known pathogens of international

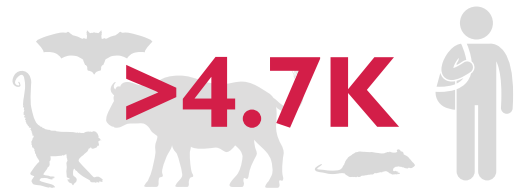
concern (e.g. MERS, Ebola, SARS-CoV-2) upon their first introduction to Thailand, and to characterize and genotype strains of known and novel viruses from people and animals. Due to its abundant experience diagnosing EID viruses over the past 10 years, PREDICT Thailand's laboratory has been designated the Ministry of Public Health's reference laboratory for identification of EIDs. The first imported MERS case in Thailand in 2015 was identified and confirmed by the PREDICT lab team in country within 24 hours after receiving the specimen. During the Ebola outbreak in Africa in 2014, a PREDICT project protocol was used to test human biological samples from suspected imported cases in Thailand during the first few months when the standard diagnostic assay was not available in country. The PREDICT project in Thailand has also facilitated collaborations both nationally (Thai National Science and Technology Development Agency (NSTDA) and Thailand Research Fund) and internationally (United States Defense Threat Reduction Agency's Biological Threat Reduction Program (US-DTRA BTRP) to achieve EID monitoring and preparedness in the country. Thailand is a success story for capacity strengthening within the PREDICT project and has become a regional leader in laboratory diagnostics and training – working with partners in Myanmar, Laos, Malaysia, Indonesia and beyond regularly exchanging information and leading joint lab and field training activities.

LOCAL PARTNERS

- WHO Collaborating Centre for Research and Training on Viral Zoonoses
- Thai Red Cross Emerging Infectious Diseases Health Science Centre
- Faculty of Medicine, Chulalongkorn University
- Faculty of Forestry and Faculty of Veterinary Medicine, Kasetsart University
- Thai Department of National Parks, Wildlife and Plant Conservation
- Thai Department of Disease Control, Ministry of Public Health
- Thai Department of Livestock and Development
- Office of Disease Prevention and Control 5
- Loei Hospital
- Health Promoting Hospital, Tambon Wat Luang
- Office of Khao Chong Phran Non-hunting Area
- Phu Luang Wildlife Research Station



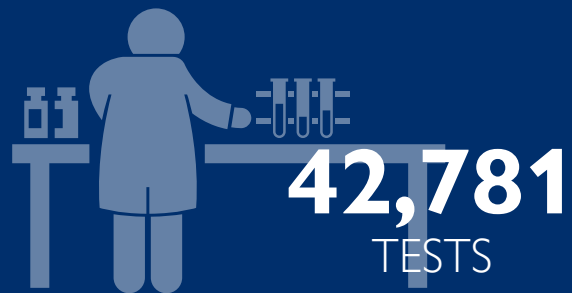
DEVELOPED the One Health Workforce by training more than 310 people in Thailand.



OPERATIONALIZED One Health surveillance and sampled over 4.7K animals and people, helping minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

· WHO-CC Viral Zoonoses Chulalongkorn University



DETECTED 152 unique viruses in both animal and human populations.



SUPAPORN

WACHARAPLUESADEE

Country Coordinator &
Laboratory Chief
WHO-CC Viral Zoonoses
Chulalongkorn

“PREDICT is a live exercise program. PREDICT is a One Health University without degree but is so much more. PREDICT is country-strengthening. PREDICT is not just a program, it promotes country’s success on EID preparedness. PREDICT is more than just predicting. Without PREDICT, Thailand may have been slow on handling the response to Ebola, MERS and COVID-19 introductions. PREDICT has helped us to gain experience, make unknown pathogens known, and build partnerships and trust.”



THANAPOL

PHUTTHARAKSA

Head of Village Health
Volunteers
Chonburi Province

“PREDICT has been very beneficial for communities. Before TRC-EID implemented the PREDICT project in our community, we were not even aware that bats harbored infectious pathogens.”

ACHIEVEMENTS

- Early detection of imported human cases of MERS and SARS-CoV-2, and screening for suspected Ebola cases, using PREDICT project diagnostic protocols.
- Trained over 300 people in core skills required for zoonotic disease surveillance and detection, including rangers and staff from the government Department of National Parks, Wildlife and Plant Conservation.
- Collected more than 36,000 specimens from >4,700 individual animals and people at high-risk interfaces throughout 10 years of PREDICT project activities.
- Discovered 102 new viruses and detected 40 previously-identified viruses in viral families with pandemic potential from bats, rodents, and primates throughout 10 years of PREDICT project activities.
- Conducted behavioral risk research to identify zoonotic disease risk factors and trained field staff from the Ministry of Public Health on best practices for in-depth behavioral risk investigations.
- Trained laboratory personnel from the Ministry of Public Health, Department of Disease Control on using PREDICT protocols for novel virus detection.
- Collaborated with the Department of National Parks, Wildlife, and Plant Conservation (DNP) in the investigation of a wildlife die-off event in Kanchanaburi Province.
- Led a health screening for the ‘Cave Boys’ rescued soccer team to test for exposure to multiple pathogens using PREDICT and other protocols to identify potential zoonoses.
- Expanded local community health engagement to include zoonotic disease awareness and prevention at multiple sites.

ONE HEALTH SURVEILLANCE



The PREDICT team conducted concurrent biological surveillance activities at high-risk animal-human interfaces in the country, focusing on in-depth 'One Health' investigations at three sites over the last 5 years. Putting One Health into action, the team successfully coordinated with local health facilities and the government Thai Department of Livestock Development (DLD) to conduct triangulated human, wildlife, and domestic animal surveillance. While the PREDICT team safely collected samples from wildlife (bats, rodents, and non-human primates) and humans in collaboration with local Ministry of Public Health and Community Health Volunteer staff. PREDICT also conducted behavioral risk investigations among healthy humans living in these same sampled communities. In partnership with the Food and Agriculture Organization (FAO), DLD staff collected samples from pigs and other domestic animals alongside PREDICT. DLD staff were then trained on PREDICT project diagnostic PCR protocols and data entry processes using the EIDITH program. This collaboration is a great example of how the project helped bring teams from different sectors together to efficiently and effectively conduct One Health disease surveillance.

The PREDICT team selected sites for One Health surveillance that included interfaces associated with land use change, wildlife consumption, guano harvesting, and human dwellings sited near large wildlife populations in three provinces of Thailand:

- *Loei Province. Loei Province is a typical provincial rural area from northeastern Thailand in the midst of land use conversion, as forest is cleared for production of rice, corn, and cassava. It is also the site of an active rodent trade, as rodent consumption is common. Bats and rodents in and around land conversion areas, human dwellings, and markets were sampled. The PREDICT team also worked with the provincial hospital in Loei to conduct surveillance of patients with hemorrhagic and encephalitic symptoms.*
- *Ratchaburi Province. Ratchaburi Province is in the western part of Central Thailand. Karst (limestone mountain) formations and caves are common, and our surveillance sites included caves where bat guano is collected by hand and sold for local agricultural production. PREDICT previously identified Betacoronavirus Group C (MERS-CoV group) viruses from bat guano collected from this region. Bat guano miners and people working in the bat guano fertilizer trade (sellers, buyers, middle men, handling workers), all high-risk occupations for disease transmission, were targeted for behavioral risk interviews and biological sample collection. Bats, rodents, and non-human primates are abundant at these sites that include tourist attractions and were also sampled as part of our effort to investigate viral sharing between the closely linked human and wildlife populations. Livestock and domestic animals were also sampled around bat roost temples and villages in collaboration with FAO and DLD.*
- *Chonburi Province. Chonburi is a province in Central/Eastern Thailand where there is extensive swine production and mixed agricultural production. Large fruit bat colonies exist in this province where Nipah virus has been previously identified. Fruit bat foraging areas overlap with pig production sites, and the Buddhist temple that harbors bat populations is a local site of worship and a tourist attraction. Fruit bats in the communities located near pig farms were sampled by the PREDICT team while pigs were sampled in collaboration with FAO and DLD partners. A local health promotion clinic, directly opposite a large fruit bat roost, was the site for human community surveillance of people living or working close to bat roosts.*

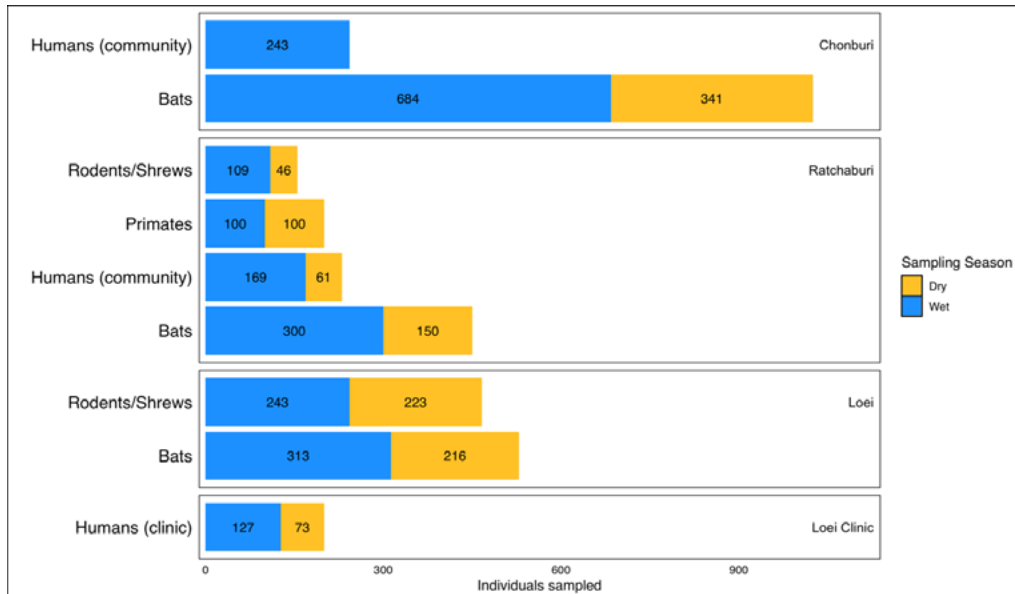


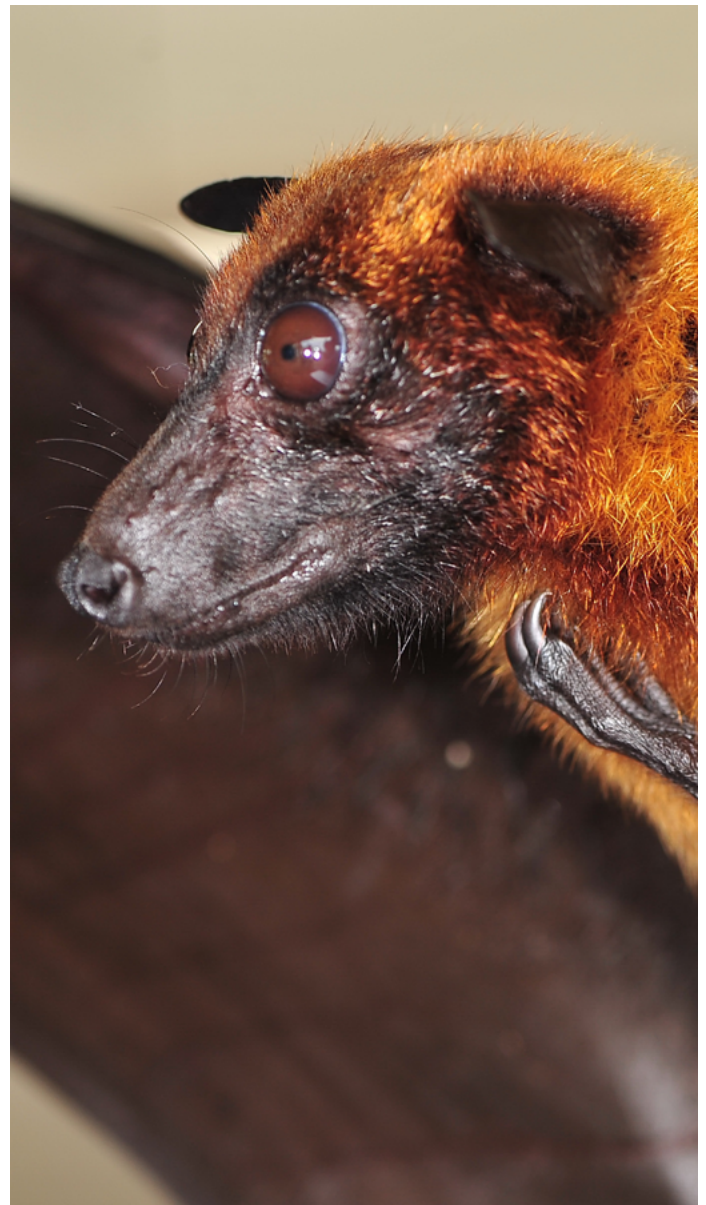
FIGURE 1. Summary of the PREDICT project's One Health sampling efforts at each surveillance site in Thailand.

VIRUS DETECTION

VIRUS FINDINGS IN ANIMALS

The PREDICT project's strategy for viral detection included screening samples using broadly reactive consensus PCR (cPCR) for priority viral families, including corona, filo, flavi, and paramyxo families, and influenza virus. Positives detected using these assays were sequenced to identify the viruses and compare their relationship to known pathogens, and viruses were prioritized for further characterization. This approach allows for detection of both known and novel viruses and improves our understanding of the presence and diversity of viruses, as well as potential pathogens, in humans and animals.

Using this approach, the PREDICT team safely tested 5,397 bat, 1,696 rodent, and 896 non-human primate oral and rectal swab samples. Of these 7,989 specimens tested, 263 were positive for coronaviruses (217 bats and 46 rodents), and 36 for paramyxoviruses (30 bats and 6 rodents). Significant coronavirus findings in wildlife included the discovery of SARS related coronaviruses in roundleaf bats (*Hipposideros* spp.); MERS-related viruses discovered at a bat guano harvesting human interface; identification of Porcine Epidemic Diarrhea virus in bats; four novel bat coronaviruses detected more than 30 times each; and a diversity of other new coronaviruses from both bats and rodents. Significant paramyxovirus findings in wildlife included multiple Nipah virus detections; several new rodent species recognized as hosts for Murine coronavirus – expanding the known host range; and detection and characterization of 15 new paramyxoviruses discovered in bats and rodents. No filoviruses, flaviviruses, or influenza viruses were detected from any wildlife specimens. For details on detections and testing effort for each viral family and host genus combination, see heatmaps and tables below.



	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
Chaerephon (bats)	3.3% (2787)	0% (1773)	0% (1773)	0% (1773)	0.5% (1773)
Cynopterus (bats)	6.7% (30)	0% (20)	0% (20)	0% (20)	0% (20)
Eonycteris (bats)	12.9% (62)	0% (12)	0% (12)	0% (12)	0% (12)
Hipposideros (bats)	9.9% (71)	0% (35)	0% (35)	0% (35)	0% (35)
Miniopterus (bats)	10.7% (28)	0% (15)	0% (15)	0% (15)	0% (15)
Myotis (bats)	4.5% (22)				
Pteropus (bats)	6.8% (2298)	0% (1498)	0% (1498)	0% (1498)	1.6% (1598)
Rousettus (bats)	0% (7)	0% (2)	0% (2)	0% (2)	0% (2)
Taphozous (bats)	0% (26)				
Canis (carnivores)	0% (153)	0% (102)	0% (102)	0% (102)	0% (102)
Felis (carnivores)	0% (156)	0% (104)	0% (104)	0% (104)	0% (104)
bos (cattle/buffalo)	0.5% (216)	0% (144)	0% (144)	0% (144)	0% (144)
Capra (goats/sheep)	0% (90)	0% (60)	0% (60)	0% (60)	0% (60)
Macaca (non-human primates)	0% (896)	0% (556)	0% (556)	0% (548)	0% (556)
Bandicota (rodents/shrews)	0% (190)	0% (125)	0% (116)	0% (125)	0% (125)
Berylmys (rodents/shrews)	33.3% (6)	0% (4)	0% (4)	0% (4)	0% (4)
Chiropodomys (rodents/shrews)	0% (3)	0% (2)	0% (2)	0% (2)	0% (2)
Leopoldamys (rodents/shrews)	1.6% (61)	0% (42)	0% (38)	0% (42)	0% (42)
Maxomys (rodents/shrews)	2% (51)	0% (34)	0% (32)	0% (34)	0% (34)
Mus (rodents/shrews)	9.2% (87)	0% (58)	0% (55)	0% (58)	1.7% (58)
Niviventer (rodents/shrews)	4.8% (21)	0% (14)	0% (13)	0% (14)	0% (14)
Rattus (rodents/shrews)	3.3% (1317)	0% (879)	0% (848)	0% (879)	0.6% (879)
Unknown (rodents/shrews)	0% (27)	0% (16)	0% (9)	0% (16)	0% (16)

FIGURE 2. Heatmaps providing a summary of sampling effort and detection of positives for the five priority viral families: coronaviruses, filoviruses, flaviviruses, influenzas, and paramyxoviruses. The data are summarized by host at the genus level, and broader taxonomic groups are shown in parentheses. The heatmap data show the percentage of viral positives that were confirmed by sequencing (%) and the number of PCR tests performed (in parentheses). The red color scales with increased viral positives. Host taxa or interfaces that did not test positive are shown in white.

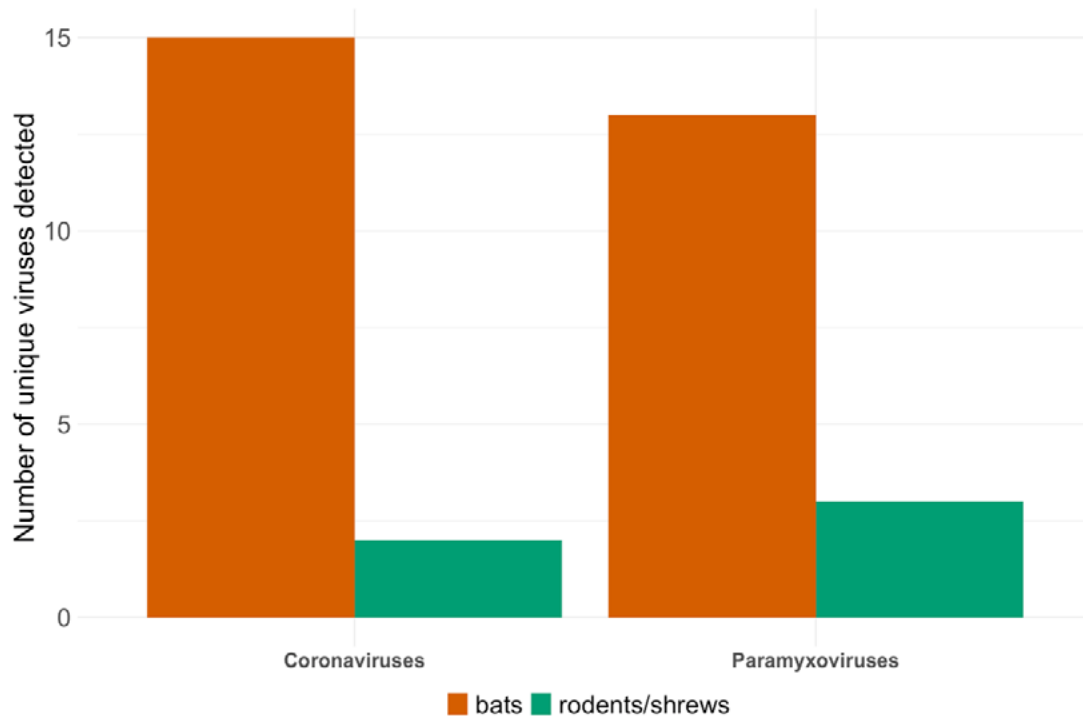


FIGURE 3. Number of unique coronaviruses and paramyxoviruses detected in bats and rodents in Thailand.

In addition to characterizing a number of newly discovered viruses from wildlife, another outcome of PREDICT sampling and testing was expanding the known host range for previously described viruses. For example, 20 new rodent host species were discovered for Murine coronavirus from the PREDICT project sampling efforts, six of these from

Thailand. Host range information is important for helping predict spillover potential of viruses, as viruses with a large “breadth” of different hosts are more likely to infect people – as was shown in previous analyses from the PREDICT project (Olival et al. 2017; Johnson et al. 2015).

VIRUS FINDINGS IN PEOPLE

Through partnerships with national and community-level public health experts the PREDICT team conducted both community and clinic-based surveillance for emerging viruses in people. Nine known human pathogens were detected during human surveillance activities, with a majority discovered during hospital and local clinic-based surveillance (Table 1). The most common virus detected was influenza virus, including both Influenza A and B strains, but notable paramyxoviruses including Human Parainfluenza virus 1 and

Measles virus were also identified from clinical specimens. Influenza A and B strains were detected in approximately 50 patients from Loei hospital, and interestingly the virus was detected in nasopharyngeal swabs and in many cases also in rectal specimens from the same individuals, or in rectal specimens only (Figure 4, Sangkakam, In Review). This finding has relevance for designing passive surveillance of human influenza strains, i.e. through non-invasive testing of sewage systems.

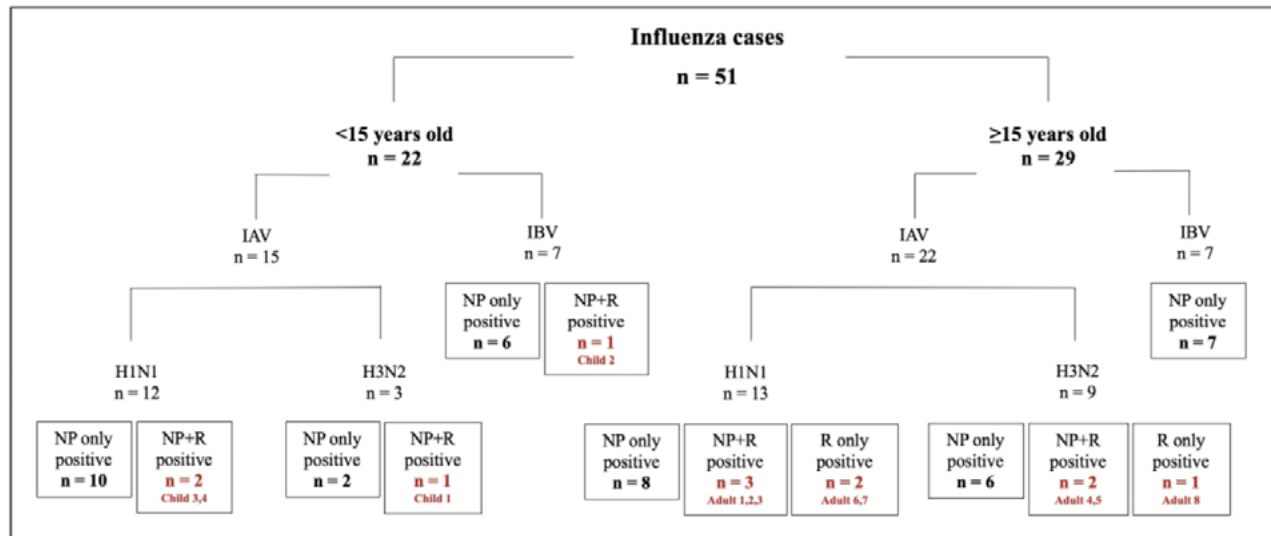


FIGURE 4. Summary of approximately 50 influenza positive specimens (IAV = Influenza A virus; IBV = Influenza B virus) from clinical surveillance at Loei hospital, showing influenza detection in both nasal (NP) and rectal (R) specimens (Sangkakam et al. In Review).

The PREDICT team provided critical support to the Ministry of Public Health (MOPH) and helped facilitate the early detection of imported emerging human viruses to Thailand. This included the rapid detection of MERS-CoV, suspected cases of Ebola virus during the West Africa outbreak, and COVID-19 in one of the earliest cases of spread out of Wuhan, China using PREDICT PCR protocols. The PREDICT and MOPH teams reported detecting the first MERS-CoV case in Thailand which was later published in the Eurosurveillance journal, August 2017. This success story highlights how using multiple coronavirus and MERS-specific assays at once allowed for rapid detection and coronavirus sequence confirmation within 24 hours, thus halting local transmission.

Other success stories from human surveillance in Thailand include supporting the MOPH's national surveillance program for hand, foot, and mouth disease. In this case, the PREDICT lab used the project's Enterovirus PCR protocols for testing patient specimens on behalf of the MOPH. Also, as part of national guidelines from the Bureau of Epidemiology, Department of Disease Control, MOPH, PREDICT project

protocols (human sampling and PCR) were used in collecting and testing samples from the youth soccer team trapped in the Tham Luang cave and their rescuers. Collected samples were tested at PREDICT laboratories (see Special Features below).

A notable finding from community-based surveillance was the detection of Human Coronavirus HKU1 (HCoV-HKU1) in a bat guano miner (harvester). The PREDICT Thailand team was able to sequence, characterize, and publish this finding in 2019 (Joyjinda et al. 2019). This virus was detected from an individual with a high level of occupational exposure to bat feces, but who was asymptomatic at the time of sampling. While this individual was likely exposed to HCoV-HKU1, a fairly common human coronavirus, due to person-to-person transmission and not via exposure from bats, our surveillance strategy and viral characterization pipeline provide valuable insight into the circulation of endemic infectious diseases in Thailand and increase the country's preparedness for other novel emerging infectious diseases.

TABLE 2. VIRAL DETECTIONS IN HUMANS BY SITE AND SPECIMEN TYPE.

VIRUS	VIRAL FAMILY/GENUS	# OF DETECTIONS	SITE	SPECIMEN TYPES
BETACORONAVIRUS 1 (OC43)	CORONAVIRUSES	2	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB
HUMAN CORONAVIRUS HKU1	CORONAVIRUSES	1	RATCHABURI	NASOPHARYNGEAL SWAB
DENGUE VIRUS	FLAVIVIRUSES	1	LOEI HOSPITAL	BLOOD (WHOLE)
ZIKA VIRUS	FLAVIVIRUSES	1	CHULALONGKORN HOSPITAL	URINE
INFLUENZA A	INFLUENZA VIRUSES	46	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB
INFLUENZA B	INFLUENZA VIRUSES	10	LOEI HOSPITAL	NASOPHARYNGEAL SWAB
HUMAN ENTEROVIRUS B	ENTEROVIRUSES	2	LOEI HOSPITAL	RECTAL SWAB
HUMAN PARAINFLUENZA VIRUS 1	PARAMYXOVIRUSES	9	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB
MEASLES VIRUS	PARAMYXOVIRUSES	2	LOEI HOSPITAL	NASOPHARYNGEAL SWAB, RECTAL SWAB



VIRUS TABLE

VIRAL FAMILY	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43)	Human	Loei Hospital	1	0	1
	Human coronavirus HKU1	Human	Ratchaburi	1	0	1
	PREDICT_CoV-16	Indian Flying Fox, Lyle's Flying Fox	Chonburi	34	31	3
	PREDICT_CoV-17	Indian Flying Fox, Lyle's Flying Fox	Chonburi	88	77	11
	PREDICT_CoV-22	Dawn Bat	Khao Chakan	7	7	0
	PREDICT_CoV-24	Greater Short-Nosed Fruit Bat	Loei	1	0	1
	PREDICT_CoV-27	Large Asian Roundleaf Bat	Khao Chakan	3	3	0
	PREDICT_CoV-41	Wrinkle-Lipped Free-Tailed Bat	Ratchaburi	1	1	0
	PREDICT_CoV-47	Wrinkle-Lipped Free-Tailed Bat	Khao Chakan, Loei, Ratchaburi	19	5	14
	PREDICT_CoV-56	Greater Short-Nosed Fruit Bat	Loei	1	0	1
	PREDICT_CoV-68	Indian Flying Fox, Lyle's Flying Fox	Chonburi	13	13	0
	PREDICT_CoV-82	Wrinkle-Lipped Free-Tailed Bat	Khao Chakan, Loei, Ratchaburi	42	40	2
	PREDICT_CoV-99	Wrinkle-Lipped Free-Tailed Bat	Ratchaburi	16	2	14
	Bat coronavirus 1	Schreiber's Long-Fingered Bat, Western Long-Fingered Bat	Khao Chakan, Loei	3	3	0
	Bat coronavirus HKU10	Large Asian Roundleaf Bat	Khao Chakan	4	4	0
	Porcine epidemic diarrhea virus	Horsfield's Myotis	Khao Chakan	1	1	0
	Rousettus bat coronavirus GCCDC1/346/356	Dawn Bat	Loei	1	1	0
	Longquan Aa mouse coronavirus	Bower's Rat	Loei	1	0	1
	Murine coronavirus	Cook's Mouse, Fawn-Coloured Mouse, Indomalayan Niviventer, Neill's Long-Tailed Giant Rat, Oriental House Rat, Polynesian Rat, Red Spiny Rat	Loei, Ratchaburi	36	28	8
	Paramyxovirus	Betacoronavirus 1 (Bovine CoV)	Domestic Cow	Ratchaburi	1	1
Human parainfluenzavirus 1		Human	Loei Hospital	8	2	6
Measles virus		Human	Loei Hospital	1	0	1
PREDICT_PMV-2		Lyle's Flying Fox	Chonburi	4	4	0
PREDICT_PMV-3		Lyle's Flying Fox	Chonburi	2	1	1
PREDICT_PMV-4		Indian Flying Fox	Chonburi	1	0	1
PREDICT_PMV-5		Lyle's Flying Fox	Chonburi	5	4	1
PREDICT_PMV-6		Lyle's Flying Fox	Chonburi	4	3	1
PREDICT_PMV-10		Indian Flying Fox, Lyle's Flying Fox	Chonburi	2	0	2
PREDICT_PMV-85		Lyle's Flying Fox	Chonburi	1	1	0
PREDICT_PMV-125		Indian Flying Fox	Chonburi	1	1	0
PREDICT_PMV-128		Lyle's Flying Fox	Chonburi	2	2	0
PREDICT_PMV-140		Lyle's Flying Fox	Chonburi	1	1	0
PREDICT_PMV-142		Lyle's Flying Fox	Chonburi	1	0	1
PREDICT_PMV-155		Wrinkle-Lipped Free-Tailed Bat	Loei, Ratchaburi	9	9	0
Nipah virus		Lyle's Flying Fox	Chonburi	6	6	0
PREDICT_PMV-20		Polynesian Rat	Loei	2	0	2
PREDICT_PMV-86		Lesser Ricefield Rat, Polynesian Rat	Loei	3	3	0
PREDICT_PMV-159		Fawn-Coloured Mouse	Loei	1	1	0
Influenza virus		Influenza A	Human	Loei Hospital	38	32
	Influenza B	Human	Loei Hospital	10	7	3
Flavivirus	Dengue virus serotype 2	Human	Loei Hospital	1	1	0
	Zika virus	Human	Chulalongkorn Hospital	1	1	0
Picornavirus	Human enterovirus B	Human	Loei Hospital	2	0	2
Total				380	296	84

EPIDEMIOLOGICAL & BEHAVIORAL RISK

The PREDICT team assessed zoonotic disease risk using a multi-pronged approach, which included analysis of ecological wildlife-human interfaces, epidemiological risk based on viral detection data in wildlife, scenario and spatial modeling, and quantitative analysis of behavioral survey data.

The PREDICT project's risk-based approach to surveillance included targeted wildlife sampling from key animal-human interfaces at each sampling site. Overall, the highest rate of viral detections at these disease interfaces for coronaviruses

and paramyxoviruses in animals occurred around human dwellings. A high percentage of coronavirus positive individuals were also sampled in the market value chain, although with more limited sampling in this setting. While the total number of coronavirus detections was relatively low in the extractive industry setting, including bat guano mining from Ratchaburi Province, this interface was important given the high level of exposure to animal excreta and the types of viruses discovered (MERS-related CoVs).

	Coronaviruses	Filoviruses	Flaviviruses	Influenzas	Paramyxoviruses
dwellings	5.2% (3702)	0% (2432)	0% (2406)	0% (2432)	1.2% (2532)
dwellings; natural areas	12.6% (87)	0% (58)	0% (55)	0% (58)	0% (58)
extractive industry	1.6% (2415)	0% (1610)	0% (1610)	0% (1610)	0.4% (1610)
market and value chain	5.2% (174)	0% (118)	0% (101)	0% (118)	0% (118)
natural areas	3.4% (2227)	0% (1277)	0% (1266)	0% (1269)	0.2% (1277)

FIGURE 5. Heatmap summarizing sampling effort and detection of positives in wildlife for the five priority viral families by disease risk interfaces (e.g. market value chain, wildlife near human dwellings, etc.) at the sampling sites. Dwellings include rural, human inhabited sites in located in close proximity to wildlife populations, in some cases those sites geographically overlap with uninhabited natural areas.

MAPS & MODELS FOR THE THAILAND VIROME PROJECT PARTNERSHIPS

To further assess risk from the PREDICT project's surveillance data and viral findings, the PREDICT Thailand team, together with global PREDICT partners, combined viral detection data with epidemiological and ecological risk analyses. Analytical reports were generated directly from the EIDITH database and were shared with country teams throughout the project to help them better understand country and regional risk

and contextualize their findings as new data came in. These analyses included geographic distribution of detected viruses, host range, viral discovery rates, comparison of risk factors across sites in a country, and prioritization of species and sites for future surveillance activities (e.g. for the Thailand Virome Project Partnership (TVPP)).

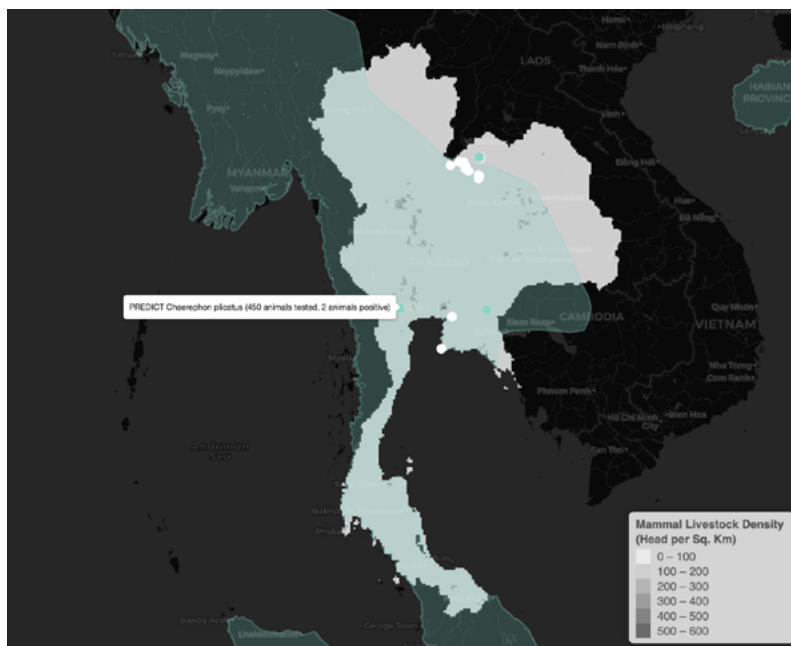


FIGURE 6. Output from the PREDICT Thailand Modeling & Analytics report showing viral data in a geographic context to explore risk assessments. Data shown here are for a novel coronavirus, PREDICT_CoV-47, with the known host species geographic range highlighted (green area) and sampling site data from PREDICT Thailand as white dots (PREDICT CoV-47 not detected) and green dots (virus was detected). Data overlaid with global data on mammal livestock density, grey-scale base layer (FAO Gridded Livestock of the World).

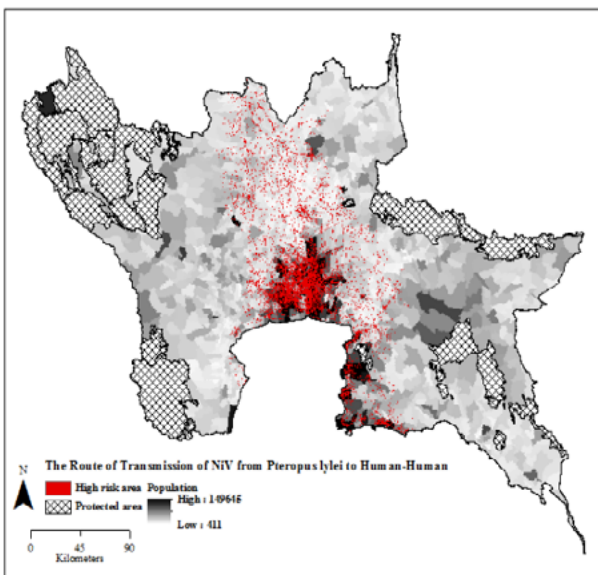
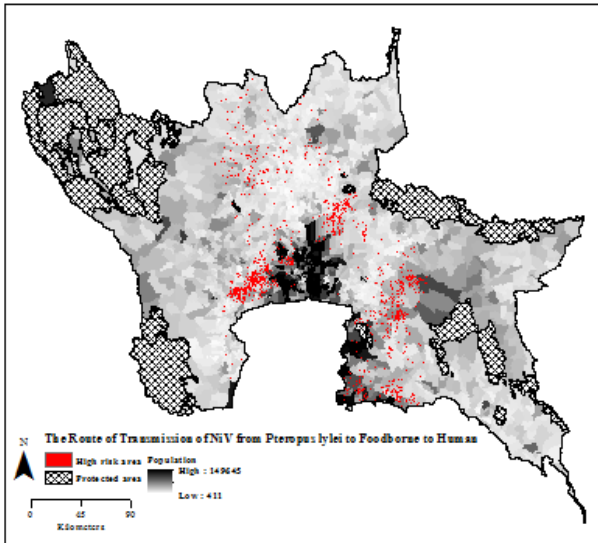
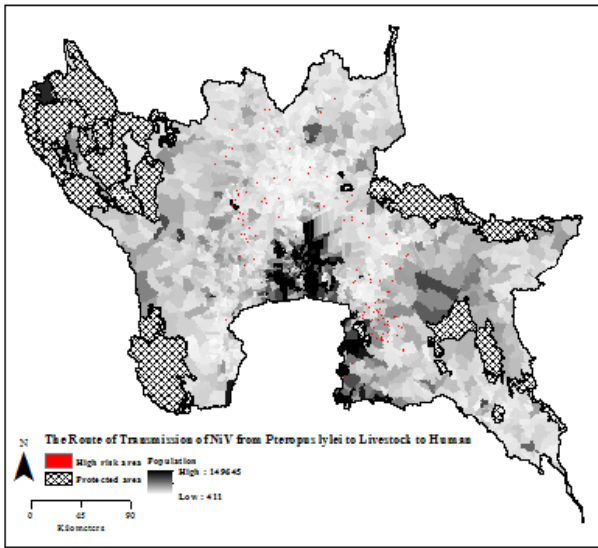


FIGURE 8. Analysis of Nipah virus spillover risk in Thailand using habitat suitability models of the primary bat host, *Pteropus lylei*, and spatial data for known drivers of virus spillover (e.g. pig density and presence of fruit orchards). Showing areas with a high risk of Nipah virus transmission from *P. lylei* to livestock (A), via foodborne (fruit orchard) sources (B), and directly to humans (C) in Thailand.

To further assess epidemiological risk from specific wildlife virus findings, we analyzed viral detection data for every virus detected by the PREDICT project in Thailand at least 30 times against various host, sampling, and ecological traits. Using a multi-variable Least Absolute Shrinkage and Selection Operator (Lasso) regression analysis, we were able to identify on the most significant variables that predict whether or not a given virus would be detected. PREDICT Thailand identified two newly discovered coronaviruses (with an unknown ability to infect people), PREDICT_CoV-17 and PREDICT_CoV-82, from 89 and 42 different animals, respectively. PREDICT_CoV-17 was found frequently in *Pteropus* spp. (large fruit bats) from Chonburi Province (Fig. 7), and PREDICT_CoV-82 was found in *Chaerephon plicatus* (cave roosting insectivorous bat) from Ratchaburi Province. Interestingly, the most important predictor for detecting both of these viruses was sampling juvenile bats. This finding, that the age structure of wildlife populations is an important risk factor, was also noted for coronaviruses in East Africa (Montecino-Latorre et al. 2020), and in global analyses for other viruses.

Nipah virus represents a clear and present threat, as it has been discovered to be circulating widely in bat populations in Thailand but has not yet been found in pig or human populations that interact with bat populations. The PREDICT team supported critical investigations into the natural ecology of Nipah virus in bats, and the risk posed to local communities living in close contact with bat populations. As part of the effort to further strengthen the capacity for zoonotic disease risk modeling in Thailand, Ms. Aingorn “Na” Chaiyes, a PhD student and member of the PREDICT team in Thailand, completed a Modeling & Analytics fellowship with EcoHealth Alliance in New York for 5 weeks in 2016. Ms. Chaiyes’ PhD research focused on using ecological niche modeling and spatial analyses tools to better understand the risk of Nipah virus spillover across Thailand. This research resulted in two peer-reviewed publications, one published and one currently in review.

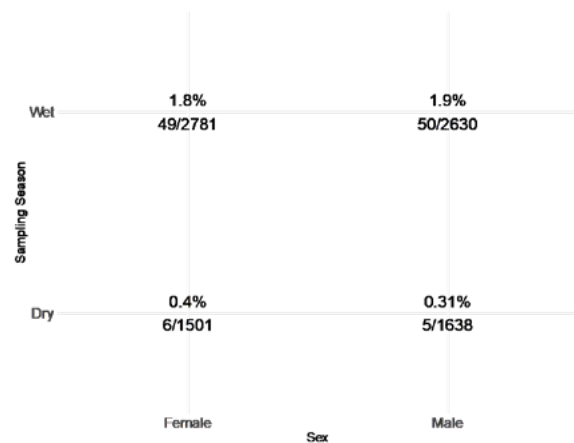


FIGURE 7. Heatplot of PREDICT_CoV-17 prevalence by sampling season and sex of bats sampled. PREDICT_CoV-17 was detected 89 times in *Pteropus* spp. (flying foxes) from Chonburi Province. No differences in prevalence were observed between male and female bats, but significantly higher prevalence was detected in wet season vs. dry season, which is likely related to the abundance of juvenile bats.

IN-DEPTH BEHAVIORAL RISK INTERVIEWS

In Thailand, behavioral risk questionnaires were administered to community members enrolled in our community virus surveillance. This included 243 participants from the concurrent (wildlife, people, domestic animals) sampling site in Chonburi Province, and 230 participants from the concurrent sampling site in Ratchaburi Province. In addition, 200 participants from Loei Province enrolled in the clinic-based surveillance were also surveyed. Some key differences in each setting were observed with implications for the frequency of animal contacts and associated risk of exposure to zoonotic pathogens, as well as other differences such as hygiene practices, health-seeking behavior, and knowledge about zoonotic risk.

In Loei Province, a more rural setting bordering mountainous areas, there was a high degree of contact with live animals in and around the home, including with rodents, and 39.5% of participants reported eating undercooked or raw meat and organs from animals. We also analyzed the specific types of contact with each wildlife taxonomic group (bats, rodents, and primates) from each site. For example, in Loei Province, 27% of participants noted rodents around their homes and 8% have slaughtered rodents for food (Figure 9). In Ratchaburi Province, relatively few individuals have knowingly or directly interacted with bats, but a few individuals did acknowledge being bitten or scratched recently. These behaviors overlap in an area where we also detected a variety of rodent-borne coronaviruses and paramyxoviruses but did not find evidence of human infection with these viruses.

There appeared to be a lack of general consensus on whether live animal markets could be cause for concern, with respondents in Chonburi and Loei being split evenly on the risk that these sites presented (with 49% and 52%, respectively, indicating that “Yes,” they were worried about diseases in live animals in markets), and respondents in Ratchaburi being just slightly more concerned than not (58%). Knowledge of the risk of zoonotic disease was somewhat limited, with between 18-46% of participants (depending on site) acknowledging that slaughtering or butchering animals with an open wound could lead to disease infection. 21% and 33% of respondents in Chonburi and Loei, respectively, indicated that they did not know if there were associated dangers in this case, and 66% of respondents in Ratchaburi reported that there would be nothing to worry about. These responses, which acknowledge uncertainty and indicate misinformation, suggest potential entry points for targeted public health messaging and behavior change communication programming – particularly for those whose occupational or cultural activities center around live markets. The opportunity in Thailand to share new information and challenge existing beliefs is further reinforced when looking at the responses among those who had reported an unusual illness in the last year: the majority of respondents across each site admitted that they did not know the origins of their illness. Together, our preliminary findings suggest that some communities, including ones with high levels of animal contact, would make good targets for continued community engagement and education campaigns about zoonotic disease transmission.

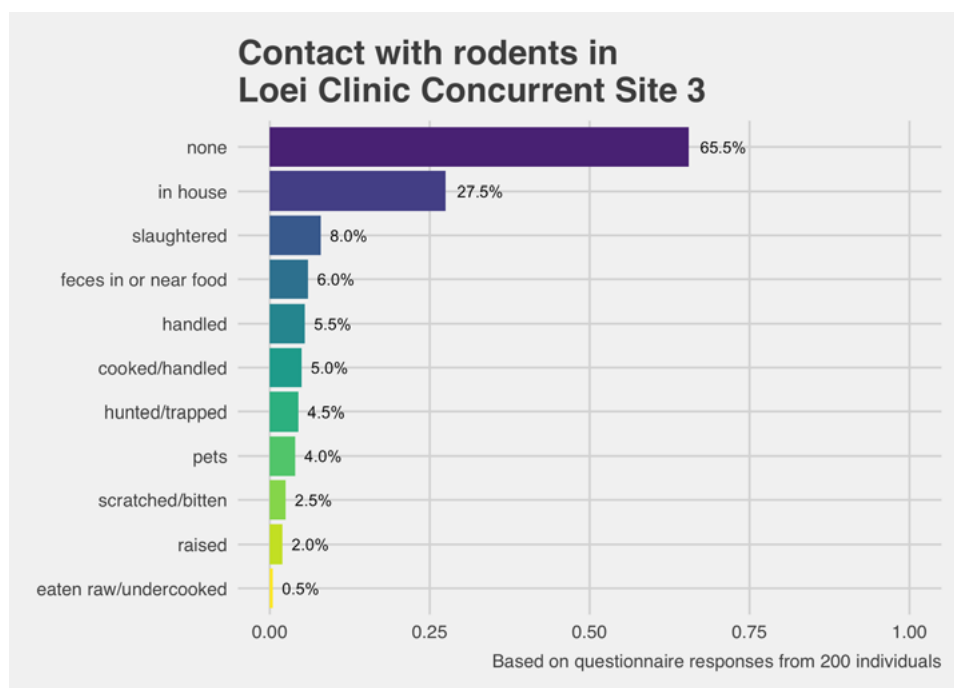


FIGURE 9. Reported human contact with rodents at various interfaces by participants from Loei Clinic.

COMMUNITY ENGAGEMENT & RISK COMMUNICATION

The PREDICT team provided summaries of surveillance and laboratory findings to the communities involved in the project. The team also shared resources and strategies with community partners designed to reduce risks for virus transmission while balancing health and conservation goals. In Ratchaburi Province, the PREDICT team conducted community outreach campaigns targeting the bat guano miner community to increase awareness and promote risk reduction strategies. The PREDICT-developed risk reduction and behavior change communication resource *Living Safely with Bats* was translated into Thai language and used to highlight ways that communities can live safely and humanely in concert with bats. More than 100 participants learned about bats, their important contributions to ecosystem services, and strategies that could reduce the risk of spillover, amplification, and spread of novel viruses. The PREDICT team also organized a “Community One Health Meeting” for villagers within Panatnikhom District, Chonburi Province on November 28, 2018 to educate them on the zoonotic diseases carried by bats and dogs. Other examples of community

engagement include organizing the “Global One Health Day” in 2017 and 2018 with government partners from 7 One Health organizations and the “Disease X conference” to disseminate research findings to the academic and layman communities, held in Bangkok on December 17, 2018.



CAPACITY STRENGTHENING



The PREDICT team was host to a large number of One Health trainings and collaborative workshops aimed at increasing capacity for pre-emptive disease surveillance and detection across the country. These trainings ranged from field, laboratory topics, and policy level collaborations with government as well as researchers and health? professionals.

The PREDICT team conducted biosafety training in bat and rodent sampling for >50 local government staff from the Department of National Parks (DNP) who worked alongside PREDICT project staff in Loei and Ratchaburi Provinces in July 2016. The team also hosted two SEAHOHUN (EPT One Health Workforce) fellows from Myanmar and Malaysia. Both individuals received training in biosafety and bat sampling.

The PREDICT project in Thailand made significant contributions to improving the capabilities of the national laboratory system in-country and in the greater Asia region by hosting workshops with FAO partners in viral detection methods and through numerous in-country events. In 2017, the team participated in the “Joint External Evaluation of IHR Core Capacity in Thailand” meeting held at the MOPH. PREDICT project protocols for broad viral screening at a family level were suggested as an alternative method to detect viruses with zoonotic potential to the committee, and examples were given to show how these new tools have been implemented in Thailand at Chulalongkorn and DLD laboratories.



The PREDICT team conducted biosafety training in bat and rodent sampling for local DNP staff involved with the PREDICT project at Loei Province on July 18, 2016. (Photo credit: PREDICT/Thailand)

PREDICT collaborated with the Department of Disease Control, MOPH to strengthen influenza surveillance and reporting. These efforts were specifically directed towards

improving the national influenza reporting system by strengthening the capacity of diagnostic laboratories at the regional level. The PREDICT team organized a hands-on training workshop, sponsored by the National Science and Technology Development Agency of Thailand, on real-time PCR diagnostics for influenza virus infection from human specimens. The workshop was organized for 14 laboratories from the Department of Disease Control in March 2017. Following the training, the PREDICT team participated in a meeting organized by the Department of Disease Control and provided expert advice on database development for influenza surveillance in Thailand.

The PREDICT project in Thailand, together with PREDICT global partners, conducted training workshops for staff from Loei hospital and the Ratchaburi Office of Disease Prevention and Control on quantitative and qualitative methods for behavioral risk surveillance and intervention development. The multi-day training involved hands-on exercises in behavioral survey collection and qualitative research, and ethical considerations for human subject research.

OUTBREAK PREPAREDNESS & RESPONSE

Due to its abundant experience diagnosing emerging viruses over the past 10 years, PREDICT Thailand's laboratory has been designated as the Ministry of Public Health's reference laboratory for identification of EIDs. At the request of the Thai Ministry of Public Health (MOPH), the PREDICT team provided technical assistance for viral detection during several noteworthy infectious disease investigations. The first imported MERS case in Thailand, in 2015, was identified and confirmed by the PREDICT lab team within 24 hours after receiving the specimen. Contact tracing was implemented immediately, and known contacts were placed in self-isolation for 14 days, including air-crew, and no outbreak occurred. During the Ebola outbreak in Africa in 2014, a PREDICT protocol was used to test human samples from suspected imported cases in Thailand during the first few months when the standard diagnostic assay was not available in country. In January 2020, the PREDICT team also detected the first human COVID-19 case in-country (see Special Features below).

In partnership with the government Department of National Parks, Wildlife and Plant Conservation (DNP), the PREDICT team also investigated the cause of death of more than 1,000 bats in Kanchanaburi Province in November 2015. As part of the investigation, the team sampled more than 53 bats in the field and tested the samples for the presence of viral RNA from six viral families at the project laboratory including corona-, paramyxo-, influenza, lyssa-, rhabdo-, and filoviruses (additional funding for laboratory assays was provided



Bat carcasses were sampled by the PREDICT Team in Nam's cave, Kanchanaburi Province, during an investigation into the cause of death of over 1,000 bats with partners at the Department of National Parks. (Photo credit: PREDICT/Thailand)

by FAO). Seven coronaviruses in fecal samples and four paramyxoviruses in urine specimens were identified; these results were confirmed by genetic sequencing. There is no evidence at this time to suggest these viruses pose a threat to human health or caused the bat die-off. All results were reported to government partners as part of the surveillance and outbreak investigation. It was concluded that the mortality event was likely due to weather (flooding within the cave) and not a viral pathogen.

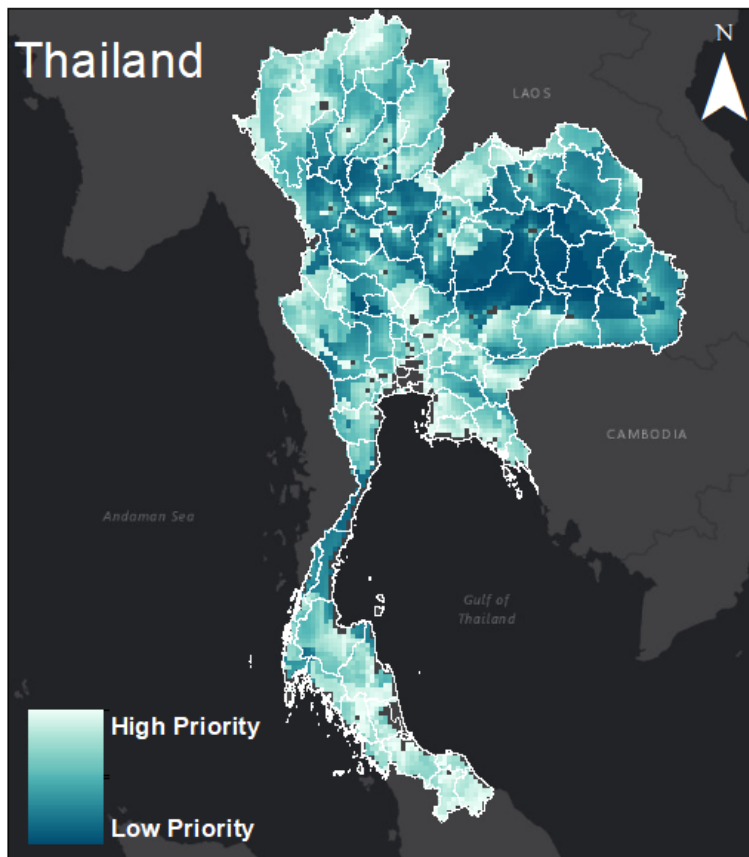
ADVOCATING FOR A GLOBAL VIROME PROJECT

On January 31st, 2017, the PREDICT team hosted a Global Virome Project (GVP) seminar at Chulalongkorn University Hospital. The seminar was part of broader efforts to garner interest in the GVP and Emerging Pandemic Threats (EPT) projects, as well as zoonotic pathogens and emerging infectious disease in Thailand. On January 29, 2018, during the Prince Mahidol Award Conference (PMAC) in Bangkok, Thailand, the PREDICT Project and the Global Virome Project (GVP) planned and successfully hosted a meeting titled “Introducing the Global Virome Project”. This was the second global in-person GVP meeting following from the kick-off meeting in Beijing, China.

To set the stage for GVP, the PREDICT project contributed to the predicted viral diversity and costs of viral discovery analyses, recently published in *Science*. Building on this work,

the PREDICT team developed a spatial modeling approach to identify priority sites for optimal viral sampling in wildlife at a 10 x 10 km resolution in countries around the world.

The first country with a completed, new GVP site selection analysis was Thailand, in preparation for a Thailand National Virome Project (also known as the Thailand Virome Project Partnership - TVPP) workshop held in Bangkok in October 2018, where the findings were presented to Thai government stakeholders. The TVPP is currently developing its research activities and is in a pilot program phase under guidance from the wildlife sector at the Department of National Parks (DNP). At present, DNP is collaborating with the PREDICT Thailand team to conduct virus testing from wildlife specimens as a pilot for the TVPP at high priority locations.



Future sampling priority areas in Thailand, ranked from most optimal (white) to least optimal (dark blue). Data analysis was conducted for the Thailand Virome Project Partnership planning meetings, including the development of interactive scalable maps. We used the Zonation Conservation Planning Software to select and rank sites of highest sampling value based on the number of unique zoonotic viruses predicted to occupy each grid cell (derived from Olival et al. 2017) and the relative cost of accessing each site from nearby cities of greater than 50,000 people (Weiss et al. 2018). We identified optimal areas for viral sampling using a decision support system for spatial planning that allocates resources (e.g. sampling costs) and prioritizes areas. The result is a ranking of all potential sampling sites in Thailand based on the highest return on investment for sampling in a particular area.



THAM LUANG CAVE RESCUE

The PREDICT team played a key role during the rescue of the boys trapped in the Tham Luang cave in July 2018, which was a multinational and collaborative effort in the global spotlight. PREDICT supported the Thai Ministry of Public Health in testing samples from the 13 survivors for diseases. Being trapped in a cave without any access to food or clean water might have compromised the immune system of the boys, increasing their susceptibility to bacterial, fungal, viral or other infections including potential bat, rodent, or insect-transmitted pathogens from the environment. PREDICT PCR diagnostic protocols were used to broadly screen for known and unknown viruses, and testing results were shared in real-time with a network of public health laboratories in Thailand. Fortunately, none of the rescued young soccer players tested positive for any pathogens related to their time in the cave.

On June 23, 2018, twelve boys from the Wild Boars soccer team and their coach set out on an adventure to explore the vast Tham Luang Nang Non cave complex (“The Great Cave of the Sleeping Lady”), near the village of Pong Pa in northern Thailand. But due to the rapid onset of monsoon rains, caverns in the cave began to flood and the team became trapped in deep caverns, separated from the entrance by miles of flooded chambers. The boys were trapped and missing for nine days before specialist rescue divers located them. Then another nine days passed before all 12 boys and their coach were rescued from the cave by the divers in a massive operation that involved more than 10,000 people, miles of hose and rope, pumps, generators, and countless supporters. The collaborative rescue was a multi-national and interdisciplinary success,

and even featured One Health expertise, as the PREDICT partner laboratory at Chulalongkorn University was requested by the Thai government and the Ministry of Public Health (MOPH) to provide technical assistance with disease screening following the team’s rescue.

Following the rescue, as the soccer team members were carefully monitored by health experts, PREDICT’s lab team provided advice to MOPH staff on safe collection of specimens for viral screening, and then prepared to test the specimens for infectious pathogens. The PREDICT project’s sampling protocols were used to collect the samples and our project’s viral detection protocols, which can identify both known and emerging viral threats, were used for screening. In addition to testing at our PREDICT lab, specimens were also shared with One Health network laboratories, including the Department of Medical Sciences, Thai and US Armed Forces Research Institute of Medical Sciences, and the Department of Microbiology and Parasitology, Faculty of Medicine, Chulalongkorn University. Lab teams simultaneously ran specific assays for targeted diseases along with the PREDICT project’s family-wide viral detection assays to ensure results within 24 hours.

The exhaustive disease screening following the cave rescue demonstrate Thailand’s proactive approach surveillance and response, especially for emerging diseases and disease risks from bats and caves. In addition, the strengths of the national laboratory system were prominent, as Thailand’s disease detection network successfully collaborated to share data and information for rapid detection and identification of disease threats.



MERS-CoV PREPAREDNESS & EARLY DETECTION

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV), caused by a coronavirus, is an emerging infectious disease that was first detected in 2012. MERS-CoV is thought to have an animal source with an ancestral origin in bats and contemporary transmission from camels to people in the Middle East. Infection may also be transmitted between humans through airborne spread or direct contact, and several outbreaks have occurred around the world from imported human cases. In June 2015, Thailand saw its first case of MERS-CoV, brought into the country by an international traveler.

As a result of prior preparedness efforts, including viral discovery for human infections as part of the PREDICT project, training on sampling for potential MERS-CoV infections, and MERS-CoV laboratory screening protocols, the country had strong capacities in place. Paired with infection control practices, intensive surveillance was rapidly implemented (e.g. temperature checks) in high-risk settings including points of entry into the country and in healthcare settings. Suspected MERS-CoV patient samples were rapidly tested (only seven hours for first results and 24 hours for confirmation) at the WHO Collaborating Center for Research and Training on Viral Zoonoses at Thailand's Chulalongkorn University (which reports to the Ministry of Health). The PREDICT team tested hundreds of potential contacts identified via contact tracing by the Ministry of Public Health from several separate MERS-CoV introductions. No secondary infections were detected in Thailand suggesting no human to human transmission occurred.

Given the limited knowledge on MERS-CoV when it first emerged, the PREDICT team leveraged its ongoing wildlife surveillance efforts with One Health partners to continue to test for coronaviruses in wildlife and domestic animals in the country to help improve understanding about this group of viruses. At the request of the Government of Thailand, the PREDICT team also hosted a training on how to safely sample camels for MERS-CoV detection in February 2014. Surprisingly, there are over 50 camels in Thailand, located at various zoos and other captive facilities, and officials wanted to be sure there was no evidence for MERS-CoV infection in these captive animals. The Department of Livestock and Development followed up after the training with sampling and testing of camels in the country.



COVID-19 DETECTION IN THAILAND

The COVID-19 infection was first confirmed at the PREDICT lab at Chulalongkorn Hospital and PREDICT project viral family diagnostic protocols were used to detect and characterize the novel coronavirus from a Wuhan tourist before the specific testing protocol from WHO was available. The finding and sequence confirmation (best matched to bat SARS-related coronavirus, accessed to GenBank on January 9, 2020) was reported to MOPH within 24 hours after receiving the specimen. On January 11st, 2020 the sequence was re-analyzed with the first whole genome sequence of SARS-CoV-2 from China, the two viruses were found to be identical for a short region of the genome. The whole genome was then characterized using next-generation sequencing technology and was found to be >99% identical to SARS-CoV-2 from Wuhan.

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