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PREDICT ETHIOPIA

One Health in action (2015-2020)

Promoting vigilance for viral spillover from wildlife to people



ETHIOPIA



Identified as part of a priority region for the emergence of new viral threats such as the Middle East respiratory syndrome coronavirus (MERS-CoV), a pathogen with origins in bats transmitted to people by camels, PREDICT/Ethiopia forged strategic One Health partnerships in 2015 between UC Davis, the Aklilu Lemma Institute of Pathobiology at Addis Ababa University, and the Ethiopian Public Health Institute. PREDICT is one of the first initiatives in Ethiopia that takes a One Health approach to address wildlife zoonoses that pose substantial threats to human, animal and economic health such as MERS coronavirus. Through consultation with partners including the Ministry of Health and the Ethiopian Wildlife Conservation Authority, One Health surveillance sites were identified in high-risk interfaces for virus spillover and spread including areas in and around Awash National Park and Bati camel holding ground. Community engagement throughout the length of the project was paramount to

ensuring the success and safety of our field teams, especially given the civil unrest and political instability in the country that affected field activities.

PREDICT put One Health into action by introducing innovative disease surveillance methods, strengthening laboratory and workforce capability, and improving capacity for surveillance and detection of zoonotic diseases and emerging viral threats. Working in support of the Global Health Security Agenda in Ethiopia, PREDICT's overall goals were to identify animal reservoirs and amplification hosts for zoonotic viruses, enhance real time disease surveillance and work force capacity, and participate in collaborative One Health platforms that reduce the risk of disease spillover, amplification, and spread. Wildlife and human samples were screened for five priority viral families suspected to be sources for new potential zoonotic pathogens impacting people. Through analysis

of project data and findings, PREDICT 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 strengthened two molecular diagnostic laboratories in country, both of which are now capable of detecting nationally recognized priority zoonoses and new and emerging viral disease threats, and which contribute to training and empowering a One Health workforce for sustained disease prevention, detection, and response. An example of regional capacity building at its finest, our partners in Ethiopia benefitted from knowledge and technical skill exchange with PREDICT teams in Tanzania and Uganda; an enduring legacy as these relationships and professional networks continue long after the project has ceased.

LOCAL PARTNERS

- Addis Ababa University, Aklilu Lemma Institute of Pathobiology (AAU, ALIPB)
- Ethiopian Public Health Institute (EPHI)
- Ministry of Health
- Awash Health Center
- Bati Health Center
- Ministry of Culture & Tourism
- Ethiopian Wildlife Conservation Authority
- Ministry of Livestock Development and Fisheries (MoLDF)
- National Animal Health Diagnostics and Investigation Center (NAHDIC)
- Food and Agricultural Organization (FAO)



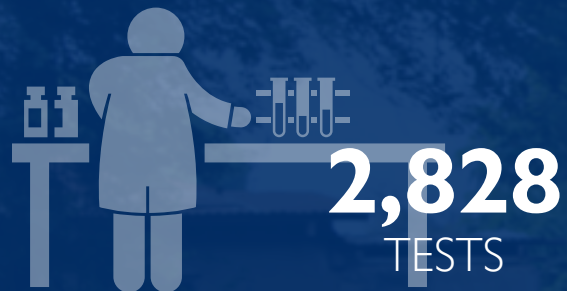
DEVELOPED the One Health Workforce by training 30 people in Ethiopia.



OPERATIONALIZED One Health surveillance and sampled over 1.3K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

· Addis Ababa University, Aklilu Lemma Institute of Pathobiology



DETECTED 11 unique viruses in both animal and human populations.



DESALEGN BELAY

Human Surveillance Lead
Ethiopian Public Health
Institute

“Prevention starts with detection, and PREDICT has brought new lab capacity and a One Health surveillance approach to prevent outbreaks.”



YOHANNES NEGASH

Wildlife Surveillance
Laboratory Lead
Addis Ababa University, Akllilu
Lemma Institute of Pathobiology

“PREDICT is one of the best coordinated projects I’ve ever seen. I have learned a lot of techniques from field sampling to laboratory testing. We have learned how to organize tasks and how to collaborate with others, and how to manage a whole project. PREDICT has taught me to follow up and harmonize all activities and to share an approach across countries. PREDICT can detect emerging and known zoonotic viruses which is very important for early warning and detection of potential pandemics.”

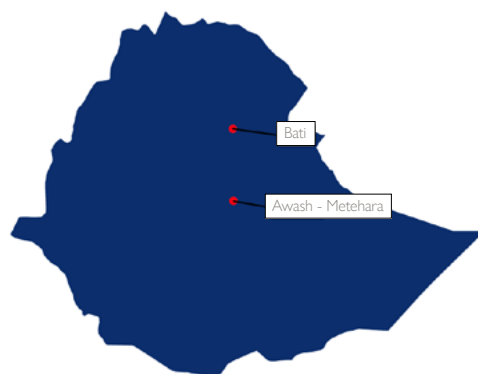
ACHIEVEMENTS

- First known project to utilize a One Health approach for zoonotic disease surveillance in bats in Ethiopia
- Implemented new field methods for wildlife disease surveillance
- Created new collaborations between universities, institutions, sectors and countries
- Established conventional PCR laboratory techniques in two laboratories in country
- Participated in the establishment of the Ethiopian National One Health Steering Committee made of representatives from EPHI, Ministry of Agriculture, Ministry of Forestry, Environment and Climate Change, four universities, EWCA, Ministry of Higher Education, and included partners from the WHO, CDC, and FAO.
- Strengthened regional networks and communication channels across East Africa
- Identified a new alphacoronavirus – PREDICT CoV-114 in 2 bat species in Ethiopia



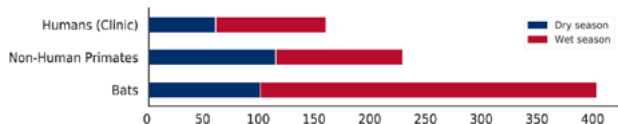
ONE HEALTH SURVEILLANCE

Concurrent sampling locations in Ethiopia were strategically chosen to reflect high-risk human-animal interfaces for virus spillover and spread. These included: areas in and adjacent to Awash National Park, in the towns of Awash and Metehara, Afar and Oromia regions; and Bati town, Amhara region, close to the Mile Serdo Wildlife Reserve. Both of these areas include growing urban centers, commercial and subsistence farms, livestock markets, and wildlife reserves. In both Awash-Metehara and Bati, wildlife sampling areas reflected a gradient of rural to urban human density impact. Syndromic (febrile) patients were enrolled at local health centers, located in the urban city center.



Awash-Metehara = bats; humans; non-human primates
Bati = bats; humans; non-human primates

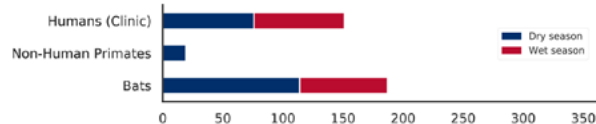
AWASH-METEHARA



Numbers of individuals sampled by taxa group in Awash-Metehara

Bats were safely and humanely sampled in a roadside cave and commercial and domestic buildings in Metehara town. Samples from bats in the roadside cave were collected from a 10mx15m volcanic blister cave adjacent to the main highway running between Addis Ababa and Djibouti. Interestingly, there is a carwash service adjacent to the cave that uses water collected from the cave floor to wash large lorries overnighting along the highway. Young men also often sleep in this cave. Bats were also sampled from a large commercial store building and a villager's residence. Non-human primates were sampled in areas in and around Awash National Park. Febrile patients were enrolled for sampling and completion of a behavioral risk questionnaire at the Awash Health Center.

BATI



Numbers of individuals sampled by taxa group in Bati

In Bati there is a camel holding ground, which is a regionally important trading point for camels moving through Ethiopia and the surrounding region. Bat sampling locations in this area included the local primary school, the Bati Health Center (where febrile patients were enrolled for sampling and behavioral risk questionnaires), and traditional styled domestic residences (hay roofed tukuls).

From 2016 – 2018, the PREDICT team safely collected and tested samples from 589 bats and 247 non-human primates during 23 sampling events over both the dry and rainy seasons. From May - October 2018, 310 febrile humans were longitudinally enrolled, sampled at the Awash and Bati Health Centers. Specimens collected from both wildlife and people included oral and nasal swabs, saliva, feces, urine, whole blood and serum.



WILDLIFE SPECIES SAMPLED AND TESTED

	2016	2017	2018	TOTAL
AWASH-METEHARA BATS	93	109	200	402
LITTLE FREE-TAILED BAT (<i>Chaerephon pumilus</i>)	60	88	80	228
LESSER MOUSE TAILED BAT (<i>Rhinopoma hardwickii</i>)	33	21	120	174
BATI BATS	0	0	187	187
MIDAS FREE TAILED BAT (<i>Mops midas</i>)	0	0	180	180
SOMALI SEROTINE BAT (<i>Neoromicia cf. somalica</i>)	0	0	7	7
AWASH-METEHARA NON-HUMAN PRIMATES	7	21	200	228
GRIVET MONKEY (<i>Chlorocebus aethiops</i>)	1	4	12	17
OLIVE BABOON (<i>Papio anubis</i>)	1	10	40	51
SACRED BABOON (<i>Papio hamadryas</i>)	5	7	148	160
BATI NON-HUMAN PRIMATES	0	0	19	19
GRIVET MONKEY (<i>Chlorocebus aethiops</i>)	0	0	19	19



T-B: Carwash service using water collected from the cave where bats were sampled in Awash-Metehara; primary school where bats were sampled in Bati

VIRUS DETECTION

PREDICT's strategy for virus detection included screening samples using broadly reactive consensus PCR (cPCR) for five priority viral families: corona, filo, flavi, and paramyxo families, and influenza viruses. Viruses detected via these assays were sequenced to investigate their relationship to known pathogens and samples were prioritized for further characterization based on these results. This approach allows for detection of both known and new viruses and improves our understanding of the potential for the virus to cause disease in humans and/or animals.

Wildlife samples were safely tested at the Addis Ababa University (AAU)/ Akilu Lemma Institute of Pathobiology

(ALIPB) beginning in 2017. Specimens from patients collected at the Awash and Bati Health Centers were safely tested at the Ethiopia Public Health Institute (EPHI) laboratory beginning in 2018. Mentoring and remote support from regional and global laboratory staff helped both laboratories successfully test samples, trouble shoot problems, and ensure test reliability. Quality assurance and control was closely monitored, with some specimens tested in parallel at UC Davis to ensure new laboratory test reliability. cPCR positive samples were sent to PREDICT's global reference laboratory at UC Davis for confirmatory testing and sequencing.

VIRUS TABLE

VIRAL FAMILY	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Betacoronavirus 1 (OC43) PREDICT_CoV-114	Human	Awash Health Center	2	1	1
		Lesser Mouse-Tailed Bat, Midas Free-Tailed Bat	Awash-Metehara, Bati	77	76	1
	Chaerephon bat coronavirus/Kenya/KY22 /2006	Little Free-Tailed Bat	Awash-Metehara	11	4	7
	Chaerephon bat coronavirus/Kenya/KY41 /2006	Little Free-Tailed Bat	Awash-Metehara	2	0	2
	Eidolon bat coronavirus	Lesser Mouse-Tailed Bat, Little Free-Tailed Bat, Somali Serotine	Awash-Metehara, Bati	3	1	2
Paramyxovirus	Human parainfluenzavirus 3	Human	Bati Health Center	1	1	0
	Measles virus PREDICT_PMV-24	Human Little Free-Tailed Bat	Bati Health Center Awash	1 2	1 0	0 2
	PREDICT_PMV-175	Midas Free-Tailed Bat	Bati	7	4	3
Influenza virus	Influenza A	Human	Awash Health Center, Bati Health Center	21	7	14
	Influenza B	Human	Awash Health Center	2	0	2
Total				129	95	34

VIRUS FINDINGS IN PEOPLE

Of the 310 humans tested, we detected and confirmed viruses in 27 patients, including: Influenza-A (n = 21), Influenza-B (n = 2), Betacoronavirus 1 – OC43 (n = 2), Measles virus (n = 1), and Human Parainfluenza virus (n = 1). More viruses were detected in people in early May and July (rainy season). EPHI partners may be pursuing further subtyping of influenza viruses.

PARTICIPANT CHARACTERISTICS ACROSS ALL SURVEILLANCE SITES

SITES	INFLUENZA A (n=21)	INFLUENZA B (n=2)	BETACORONAVIRUS 1 (OC43) (n=2)	MEASLES VIRUS (n=1)	HUMAN PARAINFLUENZAVIRUS 3 (n=1)	NEGATIVE (n=283)	OVERALL (n=310)
SEASON							
DRY	14 (66.7%)	2 (100%)	1 (50%)	0 (0%)	0 (0%)	119 (42.2%)	136 (43.9%)
WET	7 (33.3%)	0 (0%)	1 (50%)	1 (100%)	1 (100%)	164 (52.8%)	174 (56.1%)
GENDER							
FEMALE	11 (52.4%)	1 (50%)	1 (50%)	1 (100%)	0 (0%)	178 (62.9%)	192 (61.9%)
MALE	10 (47.6%)	1 (50%)	1 (50%)	0 (0%)	1 (100%)	105 (37.1%)	118 (38.1%)
AGE							
CHILD (<18)	7 (33.3%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	56 (19.8%)	64 (20.6%)
ADULT (≥18)	14 (66.7%)	2 (100%)	2 (100%)	1 (100%)	0 (0%)	227 (80.2%)	246 (79.4%)
HEALTH CENTER							
AWASH	12 (57.1%)	2 (100%)	2 (100%)	0 (0%)	0 (0%)	143 (50.5%)	159 (51.3%)
BATI	9 (42.9%)	0 (0%)	0 (0%)	1 (100%)	1 (100%)	140 (49.5%)	151 (48.7%)

VIRUS FINDINGS IN WILDLIFE

Awash-Metehara bat species sampled included Little free-tailed bat (*Chaerephon pumilus*) and Lesser mouse-tailed bats (*Rhinopoma hardwickii*); Bati bat species sampled included Midas free-tailed bats (*Mops midas*) and Somali serotine bats (*Neoromicia cf. somalica*). Out of 589 bats tested, we identified six unique viruses in 99 bats, including three known coronaviruses, two new paramyxoviruses, PREDICT_PMV-24 and PREDICT_PMV-175, and one novel coronavirus, PREDICT_CoV-114. PREDICT_CoV-114 virus is a new coronavirus found in bats belonging to the alphacoronavirus genus. Three bats exhibited viral co-infections; two bats tested positive for both *Chaerephon bat coronavirus/Kenya/KY22/2006* and PREDICT_PMV-24, and one bat tested positive for PREDICT_CoV-114 and *Eidolon bat coronavirus*. No viruses were detected in the non-human primate samples.

Bats were more likely to be positive for any virus if sampled at the Awash-Metehara site (21%) vs. Bati site (7%). Additionally,

a higher proportion of subadult age class bats were positive for any virus than adult bats of either sex, consistent across both sites. Across all sites, more viruses were detected during the rainy season (late June to September).

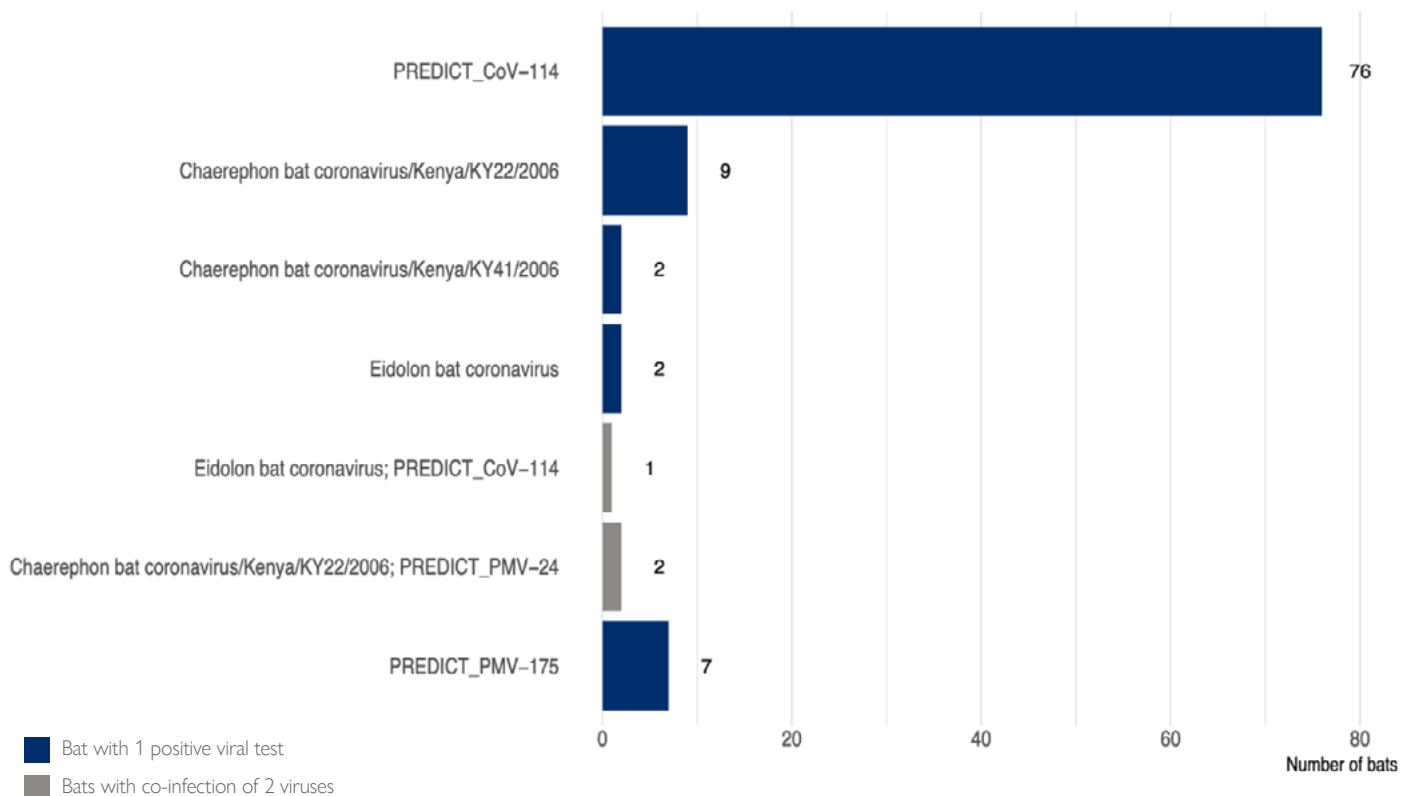
We detected the novel PREDICT_CoV-114 virus at both sites and during multiple years of sampling, with the majority of positive cases detected in August. In Awash-Metehara, this virus was detected in 72 Lesser mouse-tailed bats, during sampling trips conducted in 2016 and 2018. In Bati, this virus was found in five Midas free-tailed bats during 2018 sampling trips (only one year of sampling occurred at Bati). We detected PREDICT_CoV-114 virus in 41.4% of all Lesser mouse-tailed bats sampled. Subadult bats of both sexes were more likely to be positive for this virus than adult bats. Rectal swabs (n=49) were the most common specimen type to yield a virus sequence, though sequences were confirmed in both rectal and oral swabs collected from 23 bats, and from oral swabs collected from an additional five bats.

Our findings parallel those of a global investigation of the diversity of coronaviruses

in bats¹, with subadult bats more likely to test positive than adults, and feces/rectal swabs most likely to yield a coronavirus positive finding. However, our detection of the PREDICT_CoV-114 virus in samples collected almost exclusively during the rain season diverges from previously published results, and the lack of samples testing positive for this virus in 2017 at the Awash-Metehara site raises further questions about the epidemiology and transmission dynamics of PREDICT_CoV-114 as well as the population ecology and life history of Lesser mouse-tailed bats in Ethiopia.

There is no evidence at this time to suggest any of the viruses detected in Ethiopian wildlife pose a threat to human health. However given the recent emergence of novel betacoronavirus SARS-CoV-2, additional investigation into the ecology, evolution and global distribution of coronaviruses in wildlife is warranted. Further analysis of the alphacoronavirus PREDICT_CoV-114 identified in Ethiopia is ongoing at partner laboratories at UC Davis and Columbia University.

Viruses detected in bats





EPIDEMIOLOGICAL & BEHAVIORAL RISK

HEALTH CENTER SURVEILLANCE

Inclusion criteria for enrollment at both the Bati or Awash Health centers included children older than two and adults exhibiting a fever of 38°C or greater and symptoms consistent with a fever of unknown origin, influenza-like illness, severe acute respiratory illness, acute encephalitis or hemorrhagic fever of unknown origin. A total of 310 patients were enrolled and tested, 159 from Awash and 151 from Bati. 93% of patients resided in the town where the health center was located. Highest education level completed by participants varied, with the majority of adult participants having no formal education (35%) or only completing primary school (44%); 13% of adult participants completed secondary school, and 8% of adults obtained a college or higher education degree. Of the 310 patients, 289 had fevers $\geq 38^\circ\text{C}$ on admission, with an average fever of 38.4°C and approximately three days duration. The most common presenting symptoms at enrollment included 1) fever; 2) headache; 3) chills; 4) joint pain; and 5) inappetence. Fever and headache were the most common co-presenting symptoms. Two patients from Awash HC and eight patients from Bati HC tested positive for *Plasmodium falciparum* (malaria) based on microscopic examination of a blood smear. These patients were included in testing using the PREDICT testing protocol.

ENROLLED AND TESTED INDIVIDUALS BY AGE AND GENDER

HEALTH CENTER	AWASH (n=159)	BATI (n=151)	OVERALL (n=310)
GENDER			
FEMALE	101 (63.5%)	91 (60.3%)	192 (61.9%)
MALE	58 (36.5%)	60 (39.7%)	118 (38.1%)
AGE			
ADULT (<18)	135 (84.9%)	111 (73.5%)	246 (79.4%)
CHILD (≥ 18)	24 (15.1%)	40 (26.5%)	64 (20.6%)
AGE (YEARS)			
MEDIAN [MIN, MAX]	30.0 [3,80]	25.0 [3,60]	27.5 [3,80]

SELF-REPORTED BELIEF OF CAUSE OF ILLNESS

Of those >12 years old surveyed, multiple responses were provided for the causes of illness over the past year. Across all education levels, the top five responses included: 1) bad food or water; 2) contact with sick people; 3) don't know; 4) contact with other animals; and 5) contact with wild animals. For those with no formal education, the top cause of illness reported was "don't know."

LIVELIHOODS OF SURVEYED INDIVIDUALS

Across both sites, women enrolled were most often homemakers or engaged in activities or businesses that did not involve livestock or domestic animals. Men were more likely to be students, engaged in farming and crop production, or unemployed.



SELF-REPORTED HUMAN-ANIMAL CONTACT IN THE PAST YEAR

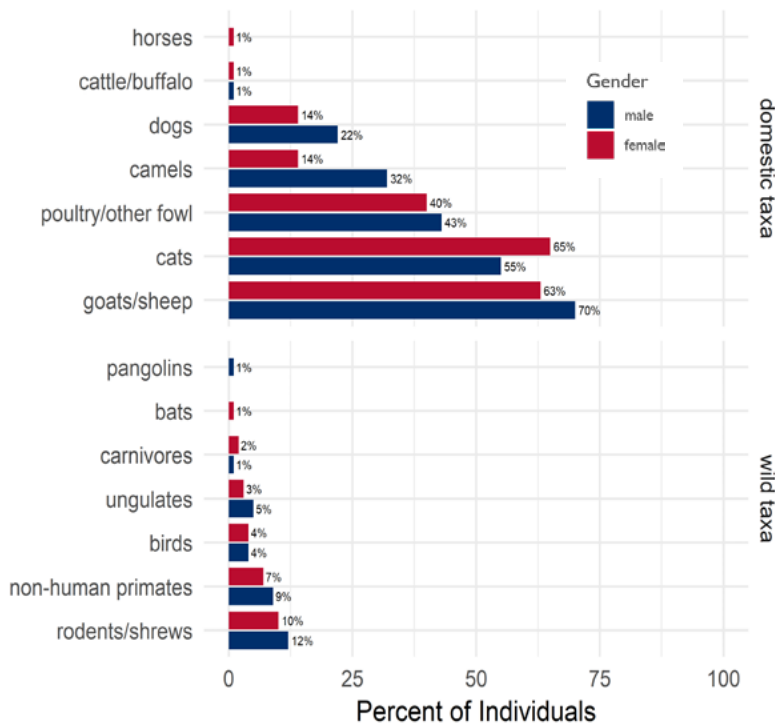
When asked about contact with animals and the type of contact experienced over the past year, rodents were the most common type of wildlife encountered by men and women, closely followed by non-human primates, a finding consistent

across both sites. No males reported contact with bats, and only 1% of women reported bat contact, especially surprising because bats living in peri-domestic locations were sampled at both sites. Both genders reported small ruminants as the most common type of livestock encountered, with men more likely to have contact with camels.

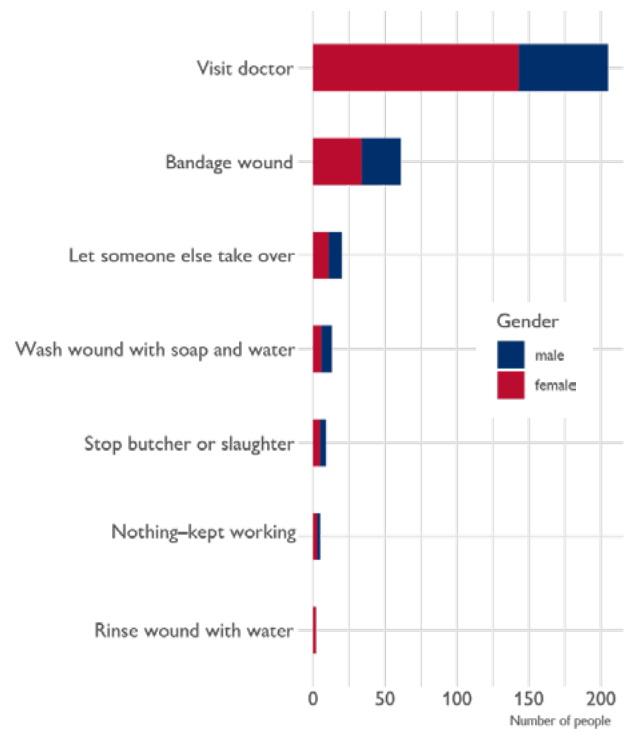
Respondents also self-reported their

behaviors following an animal scratching or biting them, or after experiencing a cut or injury when butchering or slaughtering an animal. The majority reported taking action either through visiting a doctor, bandaging or washing the wound, or letting someone else take over after such an event.

Self-Reported Human-Animal Contact in the Last Year



Action when scratched/bitten/cut





L-R: Baboon eats a banana while also providing the team with non-invasively collected saliva sample; child and baboon share play space adjacent to Awash National Park

RISK REDUCTION & COMMUNICATION

Engagement of local officials, community members and other stakeholders in the Bati and Awash-Metehara areas was a core component of project activities. Through regular meetings with community officials, our team introduced the One Health approach, and included local leaders in all steps of the project. This support was critical for obtaining local permissions, ensuring staff safety when performing sampling activities, and willingness to participate in the human surveillance portion. In addition, it was critical for gaining trust and provided a platform for sharing project findings, insights and recommendations, including successes and breakthroughs from PREDICT teams in Africa and around the world.

In Ethiopia, our team raised awareness about risk factors for zoonotic diseases in people from contact with wildlife; demographics, livelihood, and wildlife contact are all potential risk factors that could be associated with exposure to new and previously detected viruses in wildlife. We worked with communities, health centers, government officials and other stakeholders to share findings and recommend potential solutions. The high-risk interfaces we targeted in Ethiopia allowed our teams to gather information regarding community perceptions about living in close proximity to wildlife and

livestock, as well as their understanding of the risks animals pose to health. While most people did not report contact with bats, our wildlife surveillance team was frequently asked to remove bats they captured for sampling, providing the opportunity to discuss the importance of bats to the ecosystem along with ways to reduce risks and live safely with them. There remains a need and opportunity for additional education on the health and

conservation elements of living in close proximity to wildlife, particularly bats, in these areas, an initiative our PREDICT team kickstarted by distributing Amharic editions of PREDICT's behavior change and risk reduction resource *Living Safely with Bats* to government and health officials for use in community outreach and education events.²



Cover of Amharic translation of *Living Safely with Bats*



Brett Smith of UC Davis PREDICT/Global with laboratory team trained at AAU/ALIPB

STRENGTHENING CAPACITY

Our team in Ethiopia greatly benefited from the knowledge and skills of other PREDICT countries in the East Africa region, including the PREDICT teams in Uganda and Tanzania, and are the global reference labs. This regional exchange enabled the exchange of knowledge and technical skills while contributing to improved cross border collaboration and professional networking. The Ethiopia team, including professionals and faculty from ALIPB/AAU, NAHDIC and EPHI were trained on disease detection techniques and the One

Health approach to disease surveillance. In addition, staff at the Awash and Bati Health Centers all received training on safe and ethical sample collection and storage and in conducting behavioral risk interviews. Addis Ababa University staff and PhD students were trained in laboratory techniques including biosafety and biosecurity, safe and humane wildlife surveillance, and safe and biosecure sample collection and storage.

Early on in the project, core members from the Ethiopia team traveled to sites

in Uganda and Tanzania for in-depth training on wildlife surveillance and virus detection. Brett Smith from UC Davis led laboratory trainings at AAU/ALIPB and supported the establishment of a molecular diagnostic laboratory enabling the detection in country of both known and new viral threats. Additionally, Dr. Grace Mwangoka, Human Surveillance lead for PREDICT/Tanzania spent several weeks in Ethiopia supporting the training and launch of our human surveillance teams at the Awash and Bati Health Centers.



L-R: Dr. Grace Mwangoka, Human Surveillance Lead for PREDICT/Tanzania, leads a training for Ethiopian Public Health Institute staff members, Desalegn Belay, Adamu Tayachew and Mesfin Menghesha; Yohannes Negash of AAU/ALIPB training EPHI and AAU staff on cPCR techniques.



NATIONAL ONE HEALTH STEERING COMMITTEE

The One Health approach is particularly useful to bring diverse stakeholders to work together to prevent, detect and respond to increasing concerns posed by emerging public health threats. In October 2018, Ethiopia established a National One Health Steering Committee (NOHSC), improving multi-sectoral collaboration to ensure optimal health for people, animals, and the ecosystem. PREDICT/Ethiopia collaborated with partners from USAID/Preparedness and Response, USAID/One Health Central and Eastern Africa (OHCEA), the Food and Agriculture Organization (FAO), CDC, Global One Health Initiative and four Ethiopian government ministries to launch a National One Health Council. The Ministry of Health (MOH), Ministry of Agriculture and Livestock Resources (MoALR), Ministry of Environment, Forest and Climate

Change (MEFCC), and Ministry of Culture and Tourism (MOCT), signed a One Health Memorandum of Understanding with the United States Agency for International Development (USAID). PREDICT also contributed to the development of a five-year national One Health Strategic Plan that enables Ethiopia to reduce the risk of disease outbreaks and to prevent, control and respond to known and emerging infectious diseases. A National One Health Steering Committee composed of senior officials from the ministries is already in place to institutionalize and implement the plan by 2022. These successes exemplify the contributions of the PREDICT team to national Global Health Security Agenda objectives, a lasting legacy for success in disease prevention, control, and response.

PRACTICAL IMPLICATIONS

- Two laboratories, both part of the national laboratory system and representing animal and human health sectors are now empowered with the capacity and knowledge to detect known and newly emerging viral threats.
- PREDICT set a new precedent, putting One Health in action and supporting the creation of the National One Health Steering Committee and the One Health Strategic plan. These initiatives affirm the commitment of the Government of Ethiopia and national stakeholders to continue One Health work in country.
- Awash and Bati Health Clinic staff are more aware of zoonotic diseases and the importance of asking about risk factors for transmission, such as history of animal contact
- Disease surveillance in wildlife populations, particularly in bats, is an emerging frontier in Ethiopia. Our detection of the PREDICT_CoV-114 virus in Lesser Mouse Tailed bats and Midas free tailed bats is the first published findings on a new virus in bats sampled in Ethiopia.
- Additional zoonotic disease surveillance and population ecology studies of wildlife, particularly bats, in Ethiopia will be an important component of One Health surveillance work in the future

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2. *Living Safely with Bats* Amharic edition, available at <https://p2.predict.global/living-safely-with-bats-book>

For more information
view the interactive report at
p2.predict.global



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