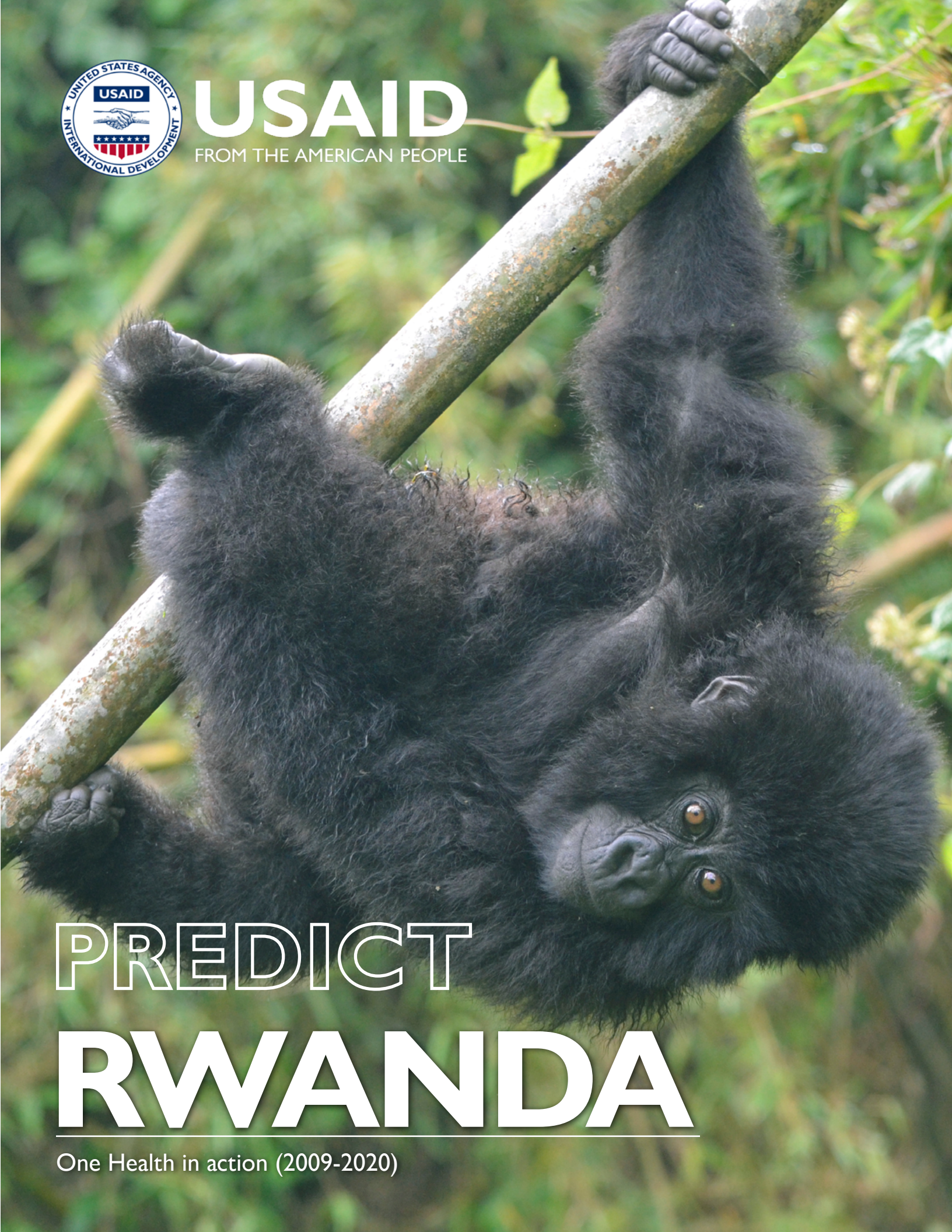

USAID
FROM THE AMERICAN PEOPLE
PREDICT
RWANDA
One Health in action (2009-2020)

Photo credit: Skyler Bishop for Gorilla Doctors

RWANDA

Rwanda is a densely populated nation in East Africa—a part of sub-Saharan Africa that is a geographic "hotspot" for emerging infectious disease. Subsistence agriculture dominates as the primary source of food and livelihoods. At the same time, Rwanda enjoys a high level of wildlife biodiversity. The Government of Rwanda prioritizes conservation through careful and intense management of protected areas that comprise about 10% of the country's total landscape, including Volcanoes National Park, the home of the endangered mountain gorillas, and Nyungwe National Park, containing 12 endemic species of non-human primate.

High biodiversity combined with high human population density creates numerous opportunities for wildlife-human interaction. In particular, mountain gorilla tourism brings thousands of people from around the world and from local communities into close contact with human-habituated mountain gorillas every day. Because gorillas share 98.5% of their genome with humans, transmission of infectious disease remains an ever-present threat to both the gorillas and people. Furthermore, wild bats and rodents in Rwanda are cosmopolitan in nature, often residing in urban centers and in human dwellings.

From 2009-2019, our team conducted field and laboratory surveillance for viral pathogens circulating in wildlife in Rwanda. In collaboration with Rwanda's Ministry of Agriculture and the Rwanda Development Board, PREDICT established the first wildlife laboratory in Rwanda, and safely screened wildlife and human samples for up to 14 viral families of concern to human health. Over 10 years, more than 2,500 wild animals (wild primates, bats and rodents) were safely and humanely captured and sampled, because these taxa have a high rate of contact with people in Rwanda, and have proven to be reservoirs for previously-known zoonotic pathogens.

As well, PREDICT/Rwanda screened 400 consenting febrile patients presenting to rural health centers neighboring Volcanoes National Park. Our team conducted questionnaires and collected biological samples to better understand the level and type of contact people have with wildlife in the region. Wildlife samples were first processed at the Wildlife Virology Laboratory, and human samples were processed at the Rwanda Biomedical Center (RBC). All samples were analyzed for the presence of virus either at RBC's National Reference Laboratory, where staff were trained on PREDICT laboratory testing protocols, or at the University of California, Davis One Health Diagnostic Laboratory.

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.

LOCAL PARTNERS

- Rwanda Development Board/Tourism and Conservation Department
- Rwanda Agriculture Board
- Rwanda Biomedical Center/National Reference Laboratory
- University of Rwanda

**Photos in this report credited to Gorilla Doctors unless stated otherwise*

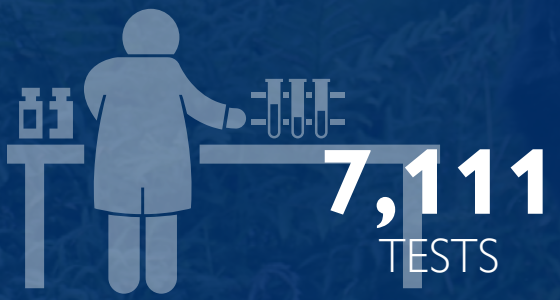
Photo credit: Skyler Bishop for Gorilla Doctors

DEVELOPED the One Health Workforce by training more than 200 people in Rwanda.

OPERATIONALIZED One Health surveillance and sampled over 2.9K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

- Rwanda Agriculture Board Wildlife Virology Laboratory
- National Reference Lab/Rwanda Biomedical Center



DETECTED 40 unique viruses in both animal and human populations.

Global Health Security Agenda

JULIUS NZIZA

Country Coordinator
Gorilla Doctors

"Implementing PREDICT in Rwanda has changed my professional life forever, and for the better. PREDICT work has been exciting in all aspects, from conducting field surveillance to engaging in laboratory work and establishing collaborations."

JEAN CLAUDE TUMUSHIME

Project Veterinarian
Gorilla Doctors

"Working for PREDICT has been a life-changing experience for my professional career in many ways. I feel proud for having been part of a team that is at the forefront of helping public health and conservation sectors design interventions that can be used to deter the next pandemic threat."

ACHIEVEMENTS

- Established the first wildlife virus testing laboratory in Rwanda. The laboratory is located in the Rwanda Agriculture Board's livestock health facility, thereby enhancing Rwanda's commitment to One Health and its focus on building One Health platforms for coordinated human and animal health surveillance and threat mitigation.
- Detected 21 novel viruses and 19 documented known viruses at critical wildlife-human interfaces in Rwanda. This information was shared with government partners to benefit both wildlife conservation and public health.
- Detected several known and novel coronaviruses in bats—a family of viruses known for causing recent emerging infectious diseases like SARS, MERS, and the current COVID-19 pandemic.
- Documented the "reverse" transmission of zoonotic pathogens from people to wildlife (gorillas).
- Interviewed 400 people to better understand the social and behavioral factors associated with zoonotic disease transmission risk.

ONE HEALTH SURVEILLANCE

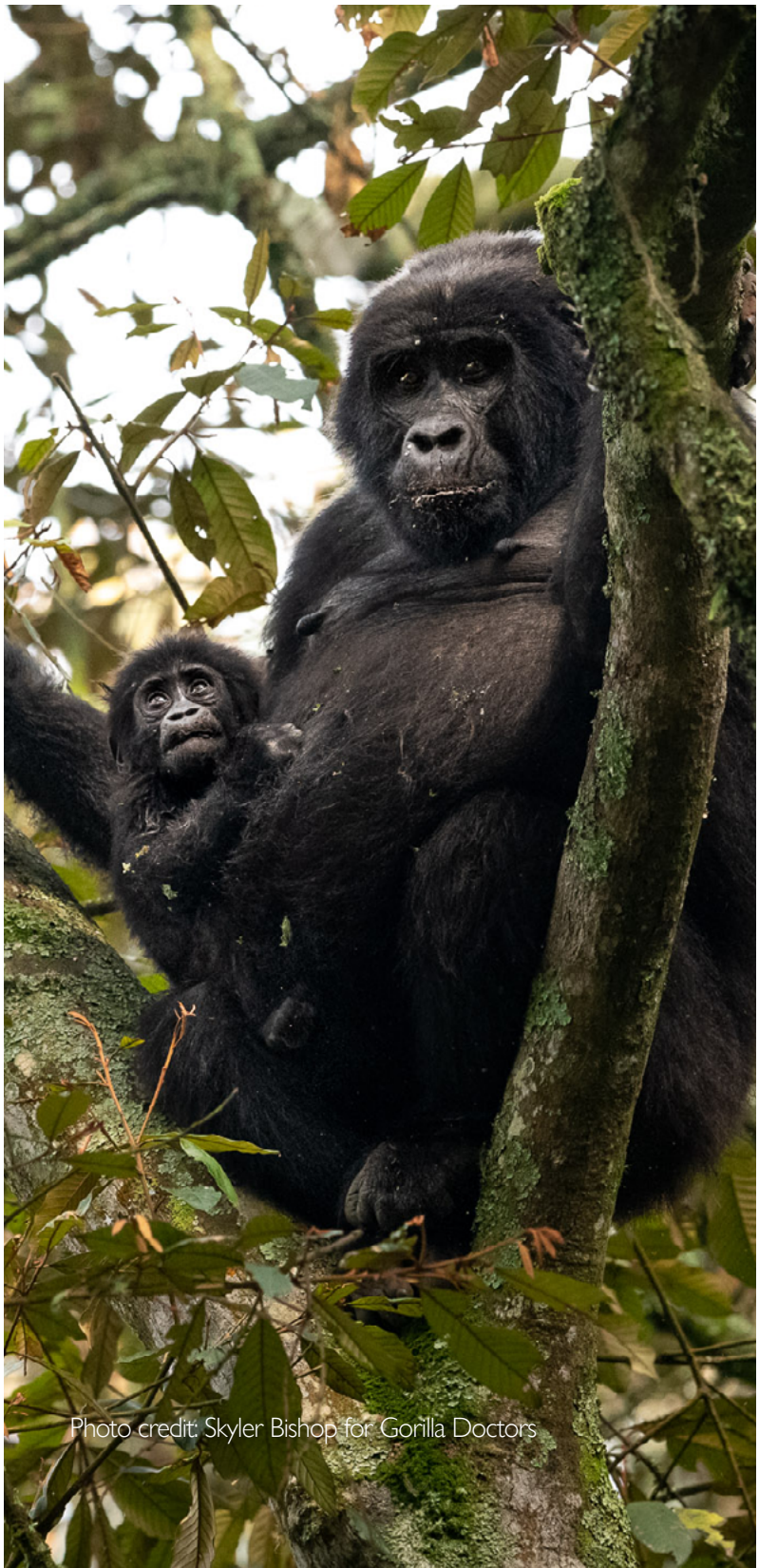


Photo credit: Skyler Bishop for Gorilla Doctors

Rwanda is a densely populated nation in sub-Saharan East Africa that is a geographic "hotspot" for emerging infectious disease. Subsistence agriculture dominates as the primary source of food and livelihoods. At the same time, Rwanda enjoys a high level of wildlife biodiversity, and the Government of Rwanda prioritizes conservation through careful and intense management of protected areas that comprise 10% of the country's total landscape, including Volcanoes National Park, the home of endangered mountain gorillas. High biodiversity combined with human population density creates numerous opportunities for wildlife- human interaction. In particular, mountain gorilla tourism brings thousands of people from around the world and from local communities into close contact with human-habituated gorillas every day. Because gorillas share 98.5% of their genome with humans, human-borne infectious disease remains an ever-present threat to this population. Furthermore, wild bats and rodents in Rwanda are cosmopolitan in nature, often residing in urban centers and in human dwellings, and bat roosting caves are gaining in popularity as a wildlife tourism destination.

In Rwanda, PREDICT has built an evidence base for priority zoonoses and emerging diseases at the human-wildlife interface. PREDICT focused its work in local communities surrounding Volcanoes National Park where there is a high-risk for human-wildlife contact. And working closely with government partners, PREDICT helped build capacity for recognition of zoonotic diseases and potential transmission pathways, supporting Rwanda's One Health workforce to prepare for emerging infectious disease prevention and control.

WILDLIFE SURVEILLANCE

In Rwanda, Volcanoes National Park and its surrounding communities is a site where people and wildlife come into daily close contact through wildlife tourism and subsistence farming. The Virunga Massif region of central – east Africa is among the most densely human-populated areas on the continent, and is further unique as a One Health site in that thousands of people come from around the world every year for the opportunity to view human-habituated mountain gorillas daily. Subsistence farming directly abuts the park boundary, and wildlife (e.g. gorillas, other nonhuman primates, buffalo) exit the park to feed in crop lands.

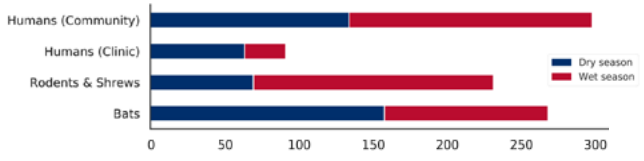
To identify viruses circulating in wildlife that have the potential to infect people, PREDICT surveillance efforts focused primarily on nonhuman primates, bats and rodents entering or living in human dwellings and accessing agricultural grounds and crops in communities adjacent to Volcanoes National Park. In addition, bats roosting in caves utilized for tourism were a focus for surveillance.

A total of 430 wild bats (insectivorous and frugivorous) were humanely live-captured in mist nets set up in villages and near roosting caves, and 286 wild rodents were humanely live-trapped in and around human dwellings and croplands. Non-human primates (n=25) were sampled opportunistically when clinical interventions were required to treat injured or ill human-habituated mountain gorillas, or when other primates (e.g. golden monkeys) exited the park and were encountered in public buildings (e.g. schools), dwellings, or crop lands.

HUMAN SURVEILLANCE

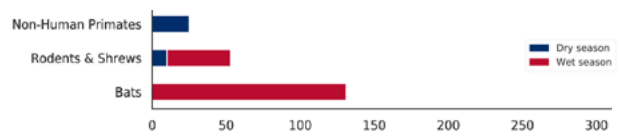
Concurrently, over a 7-mo period between December 2016 and July 2017, people living in the communities bordering Volcanoes National Park where wildlife sampling was conducted, and who presented to local community health centers with fevers of unknown origin, were included in the PREDICT One Health surveillance effort. Community health centers in which patients were sampled were located in the villages of Kinigi, Shingiro, and Bisate, and at Ruhengeri Regional Hospital in Musanze (Northern Province), all of which serve a wide-ranging community outside of the villages where the health centers are located. Patients who gave their consent for enrollment in the surveillance program provided biological samples (blood, mucosal swabs) and answered questionnaires about their demographics, livelihoods, and contact with animals (domestic and wild). Children (under the age of 18) were only enrolled with parent or guardian consent at the health centers. A total of 400 people were sampled and surveyed, including 123 adult men, 207 adult women, and 70 children.

MUSANZE REGION



Numbers of individuals sampled by taxa

NYUNGWE NATIONAL PARK REGION



Numbers of individuals sampled by taxa

VIRUS DETECTION

A subset of samples from wildlife were first processed (nucleic acid extracted) at the Wildlife Virology Laboratory at the Rwanda Agriculture Board's Rubirizi facility (Kigali, Rwanda), and then both extracted and raw (unextracted) wildlife samples were shipped to the One Health Institute Diagnostic Laboratory at the University of California, Davis (USA), where they were tested for coronaviruses, paramyxoviruses, filoviruses, flaviviruses, and influenza viruses using a broad-based consensus PCR platform. All human samples were tested at the National Reference Laboratory at the Rwanda Biomedical Center (Kigali, Rwanda), also for coronaviruses, paramyxoviruses, filoviruses, flaviviruses, and influenza viruses, using the same broad-based consensus PCR platforms. In all cases, initial results from PCR screening were confirmed via genomic sequencing in the USA. Test results were shared with the Government of Rwanda through the appropriate ministries and departments and can be viewed at **www.data.predict.global**

VIRUS TABLE (2015-2019)

VIRAL FAMILY	VIRUS	SPECIES	SAMPLING LOCATION	# OF POSITIVE INDIVIDUALS		
				TOTAL	WET SEASON	DRY SEASON
Coronavirus	Coronavirus 229E (Human strain)	Human	Bisate Health Center (Musanze), Ruhengeri Hospital (Musanze), Shingiro Health Center (Musanze)	6	0	6
	Human coronavirus HKU1	Human	Shingiro Health Center (Musanze)	2	0	2
	PREDICT_CoV-44	Angolan Fruit Bat, Sundevall's Roundleaf Bat	Nyamagabe	7	7	0
	PREDICT_CoV-77	Angolan Fruit Bat	Nyamagabe	1	1	0
	PREDICT_CoV-97	Egyptian Fruit Bat	Musanze	2	0	2
	Bat coronavirus HKU9	Egyptian Fruit Bat	Musanze	1	0	1
	Chaerephon bat coronavirus/Kenya/KY22/2006	Giant Mastiff Bat	Musanze	5	1	4
	Coronavirus 229E (Bat strain)	Angolan Fruit Bat, Sundevall's Roundleaf Bat	Nyamagabe	2	2	0
	Eidolon bat coronavirus	Straw-Coloured Fruit Bat	Kigali, Nyamagabe	13	1	12
	Kenya bat coronavirus/BtKY33/2006	Unidentified Miniopterus Bat	Nyamagabe	1	1	0
	PREDICT_CoV-94	Black Rat	Musanze	1	1	0
Paramyxovirus	Human parainfluenzavirus 1	Human	Kinigi Health Center (Musanze)	1	0	1
	Mumps virus	Human	Kinigi Health Center (Musanze)	1	1	0
	PREDICT_PMV-56	Straw-Coloured Fruit Bat	Kigali	1	0	1
	PREDICT_PMV-179	Egyptian Fruit Bat	Musanze	1	1	0
Influenza virus	Influenza A	Human	Kinigi Health Center (Musanze)	5	0	5
	Influenza B	Human	Bisate Health Center (Musanze), Kinigi Health Center (Musanze)	3	2	1
Total				**53**	**18**	**35**

VIRUSES DETECTED IN HUMANS

We detected viral RNA in 5% (18/392) of human patients sampled and tested for priority viral families. Eight patients were positive for coronaviruses, including a strain of the Human coronavirus HKU1 (n = 2 patients) and the known alphacoronavirus Human Coronavirus 229E (n = 6 patients): both of these coronaviruses are known to cause respiratory illness in people. We detected influenza viruses in eight patients, including influenza A in five individuals, as well as influenza B in three children. We also detected known paramyxoviruses, including mumps (n = 1 patient) and Human parainfluenzavirus 1 in one adult patient.

VIRUSES DETECTED IN WILDLIFE

We tested 430 bats, 286 rodents, and 25 non-human primates. We detected viral RNA from five known and three novel coronaviruses in 31 individual bats (*Eidolon helvum*, n = 13; *Hipposideros caffer*, n = 4; *Lissonycteris angolensis*, n = 4; *Otomops martiensseni*, n = 5; *Rousettus aegyptiacus*, n = 4); and *Miniopterus sp.*, n = 1). The novel bat coronaviruses PREDICT_CoV-77 and PREDICT_CoV-44 are both in the Betacoronavirus genus, which is known to include viruses of potential public health significant, such as SARS, MERS and SARS CoV-2, the cause of COVID-19. However, these new viruses detected in bats in Rwanda are not closely related to SARS or MERS. Therefore, at this time, there is no evidence to suggest these viruses pose a threat to human health. As well, the novel bat coronavirus, PREDICT–CoV-97 is in the alphacoronavirus family, and has also been found in bats in Cameroon and Tanzania. There is no evidence at this time to suggest that this virus poses a risk to human health.

For most of the known bat coronaviruses detected in Rwanda bats – Coronavirus 229E (bat strain), Chaerephon bat coronavirus/Kenya/KY22/2006, Kenya bat coronavirus BtKY33/2006, Eidolon bat coronavirus, and Bat coronavirus HKU9 – there is no evidence to suggest that these viruses pose a threat to human health.

A novel paramyxovirus, called PREDICT_PMV-56, was detected in one individual bat (*Eidolon helvum*): this virus is in the Rubulovirus genus, and there is no evidence at this time to suggest it poses a threat to human health. Three individual bats were co-infected with multiple viruses: two bats (*Hipposideros caffer* and *Lissonycteris angolensis*) were each co-infected with coronavirus 229E Bat strain and PREDICT_CoV-44, and one bat (*Eidolon helvum*) was co-infected with a known strain of Eidolon bat coronavirus and a novel paramyxovirus (PREDICT_PMV-56).

A novel coronavirus in the alphacoronavirus genus (PREDICT_CoV-94) was detected in one rodent (*Rattus sp.*). There is no evidence at this time to suggest this virus poses a threat to human health.

We did not detect virus in any of the 25 non-human primates tested.

Photo credit: Skyler Bishop for Gorilla Doctors

EPIDEMIOLOGIC & BEHAVIORAL RISK

Human surveillance conducted at Ruhengeri Hospital and the health centers in Kinigi, Bisate and Shingiro included both biological sampling, as described above, as well as qualitative and quantitative behavioral data collection in patients presenting with fevers of unknown origin. From December 2016 to July 2017, 400 patients participated in clinic-based surveillance and were administered demographic and behavioral questionnaires for syndromic surveillance purposes.

EDUCATION & DISEASE AWARENESS

Of the 355 patients over the age of 12 that reported illness in the previous year, 34% of individuals stated that they did not know the cause of their illness or sickness. Of those, 20% had no formal education, 55% completed primary school, and 25% completed secondary school. Twenty-five percent (25%) of respondents said the cause of their illness was contact with sick people, and of those, 10% had no schooling, and 44 and 46% had completed primary and secondary school, respectively. Bad food or water as a reason for illness was the response offered by 24% of those surveyed, of which 17% had no schooling, while 55% had completed primary school, and 28% secondary school. Other responses included climate change (6%) and bad spirits or witchcraft (2%) as causes of illness. Approximately 1% stated that contact with wild and domestic animals could be a cause of their illness.

LIVELIHOODS

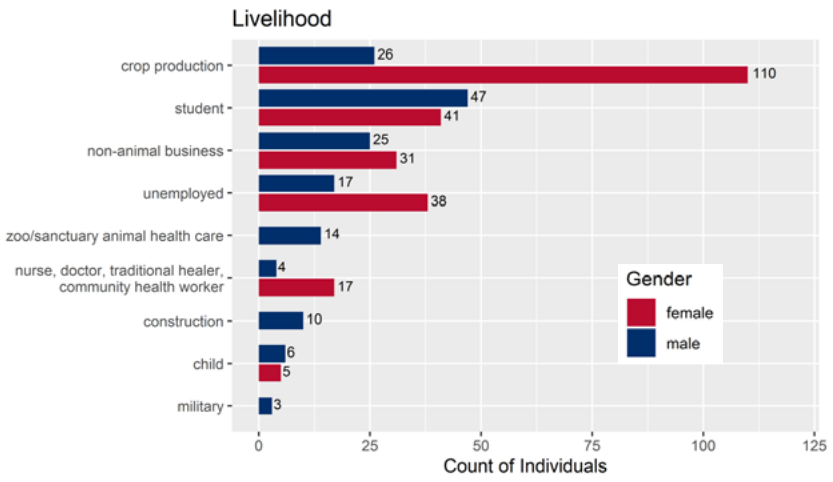
A majority of the 245 females interviewed were engaged in crop production (45%) for their livelihoods; 17% were students, 16% were unemployed, and 13% were engaged in non-anima-relatedl businesses. Of 155 males interviewed, most were students (30%), engaged in crop production (17%), or in non-animal related businesses (16%).

CLINIC-BASED SURVEILLANCE PARTICIPANT CHARACTERISTICS (n=400)

SITES	FEMALE	MALE
Bisate Health Center	61	39
Kinigi Health Center	60	40
Ruhengeri Hospital	54	46
Shingiro Health Center	70	30

DEMOGRAPHICS OF HUMANS INTERVIEWED IN MUSANZE (n=400)

	NUMBER OF INDIVIDUALS
GENDER	
FEMALE	245 (61.2%)
MALE	155 (38.8%)
AGE GROUP	
ADULT (>18 YEARS OLD)	330 (82.5%)
CHILD	70 (17.5%)
AGE	
MEAN (SD)	30.4 (16.0)
MEDIAN [MIN,MAX]	28.0 [2.10, 97.0]



SYMPTOMS

Across all enrolled patients, the average days with fever prior to presentation at the clinic was 4.2 days. The most common symptoms in addition to fever were: headache (86%), cough (76%), cold (52%), sore throat (48%), and chills (46%). Females were more likely to report fever with headache (odds ratio (OR) = 2.06) and fever with cough (OR = 1.89) than males. There were no statistical differences between males and females for other symptoms.

REPORTED CONTACT WITH WILD ANIMALS

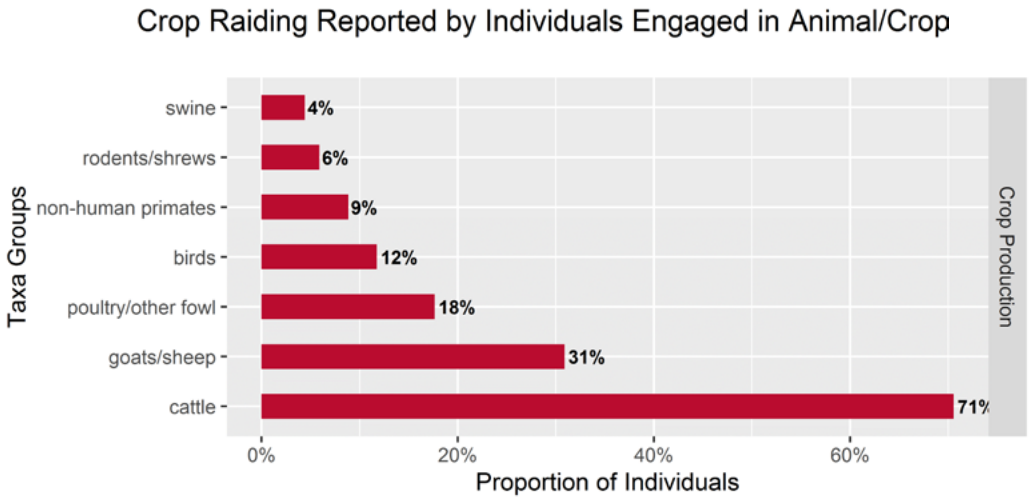
Contact with wild animals was primarily reported as keeping these animals as pets and included groups such as rodents/ shrews, bats, birds, non-human primates, carnivores, and pangolins. A majority of the 245 females interviewed were engaged in crop production (45%) for their livelihoods; 17% were students, 16% were unemployed, and 13% were engaged in non-animal-related businesses. Of 155 males interviewed, most were students (30%), engaged in crop production (17%), or in non-animal related businesses (16%).

REPORTED CONTACT WITH DOMESTIC ANIMALS

Both males and females reported contact with a variety of domestic animals, including goats and sheep, cattle, poultry and other fowl, and pigs. For both goats, sheep and cattle, the most common forms of contact were cooking or handling, eating raw or uncooked meat, and raising and handling live animals. More males (15%) than females (1.2%) reported slaughtering goats and sheep. Similarly, only males reported slaughtering cattle (6% of male respondents). Both genders reported poultry and other fowl to be living in their home, and finding poultry feces in and around their food supply.

LIVELIHOOD-SPECIFIC ANIMAL CONTACT

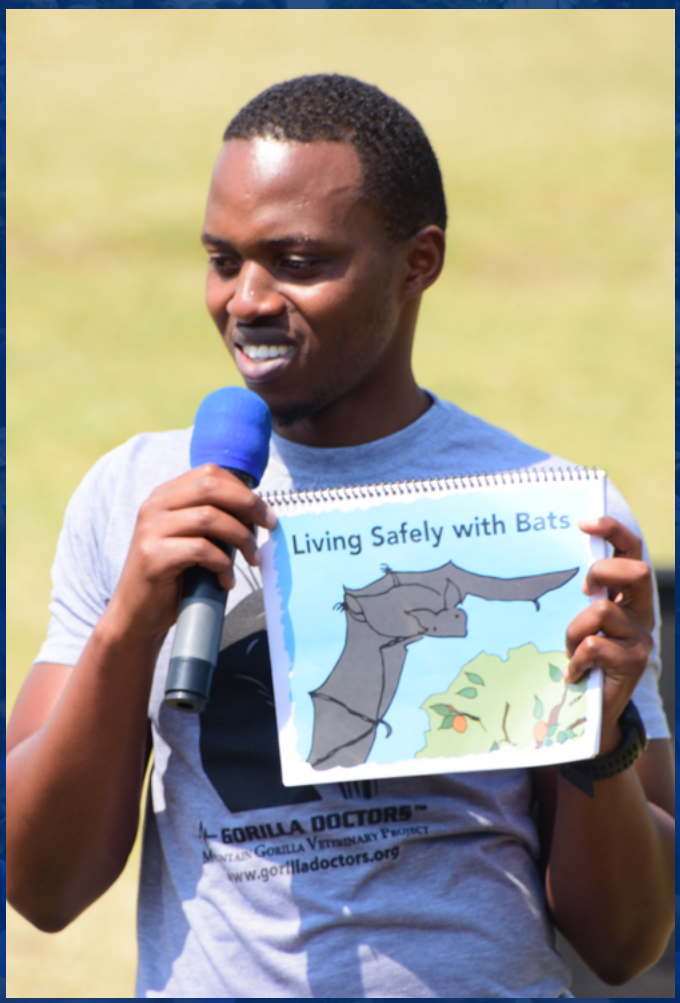
Of the 136 patients who engaged in crop production for their livelihoods, all reported that domestic and wild animals raided food supplies or destroyed crops. Cattle were most commonly reported as crop-raiders (71% of respondents), followed by goats and sheep, poultry, and wild birds. Individuals also reported crop-raiding by other wildlife taxa, including non-human primates (9%) and rodents (6%).

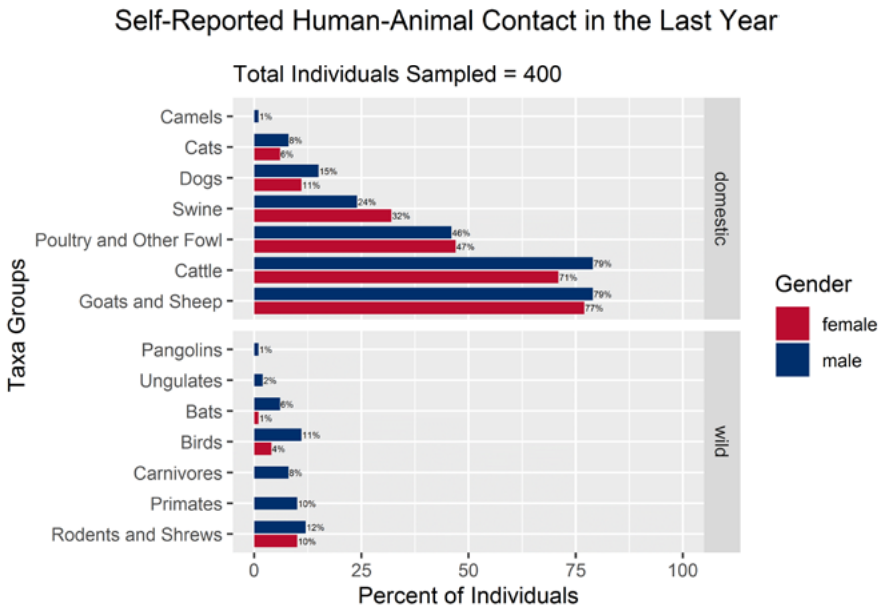


RISK COMMUNICATION

Prior to and during field surveillance, PREDICT conducted community outreach in all the district sectors where it implemented its activities. Outreach activities centered on interactions with local leaders about the project and its purpose. At the same time community members residing near the surveillance sites were sensitized about the different surveillance activities that were being conducted by PREDICT in that area, in order to foster harmony and good cooperation among project personnel, local leaders, and community members. Using the PREDICT booklet *Living Safely with Bats*, PREDICT initiated and is continuing to conduct community outreach to promote behavioral change for risk reduction in the local communities living near Musanze Caves where PREDICT conducted surveillance of roosting bats for viral pathogens.

As well, PREDICT participated in what fortunately turned out to be a "false alarm" response during an avian influenza (AI) alert in 2017. Due to the reported AI outbreak in Uganda, the Rwanda Agriculture Board (RAB) conducted massive sensitization about the disease, which led to many communities reporting on potential disease occurrence in birds at the local level. PREDICT joined RAB in responding to a report of dead wild birds in Bugarama, Rusizi District, bringing bird sampling equipment and supplies (no dead birds were observed at the site). PREDICT worked with government partners to continue sensitization about the disease in the Western and Northern Provinces via radio address.




Self-Reported Human-Animal Contact in the Last Year
Total Individuals Sampled = 400
Taxa Groups
Camels
Cats
Dogs
Swine
Poultry and Other Fowl
Cattle
Goats and Sheep
Pangolins
Ungulates
Bats
Birds
Carnivores
Primates
Rodents and Shrews
domestic
wild
1%
8%
6%
15%
11%
24%
32%
46%
47%
79%
71%
79%
77%
1%
2%
6%
1%
11%
4%
8%
10%
12%
10%
0
25
50
75
100
Percent of Individuals
Gender
female
male

STRENGTHENING CAPACITY

PREDICT Rwanda established the first wildlife virus testing laboratory in Rwanda and collected wildlife samples from major taxa for infectious diseases surveillance. The laboratory is located in the Rwanda Agriculture Board's livestock health facility, thereby enhancing Rwanda's commitment to One Health and its focus on building One Health platforms for coordinated human and animal health surveillance and threat mitigation.

PREDICT Rwanda discovered novel viruses and documented known viruses at critical wildlife-human interfaces in Rwanda. This information was shared with government partners to benefit both wildlife conservation and public health aspects.

Having established a Biosafety Level 2+ wildlife virology laboratory, PREDICT trained both wildlife and human laboratory personnel on consensus PCR techniques to analyze samples for novel and known viral pathogens. In addition, the team was trained on laboratory biosafety and contamination control by University of California, Davis research laboratory technicians.

As well, using PREDICT modules, PREDICT trained in-service personnel from the Government of Rwanda's animal and human health sectors and University of Rwanda veterinary students on Biosafety, Personal Protective Equipment use, Laboratory Biosafety, and Animal Capture techniques for bats, non-human primates and rodents.

Lastly, PREDICT Rwanda was uniquely positioned to help develop new tools for non-invasive sample collection from wild non-human primates, with application to Rwanda's mountain gorilla populations an opportunity to optimize and show proof of concept of these tools.

Photo credit: Skyler Bishop for Gorilla Doctors

PRACTICAL IMPLICATIONS

There is now ample evidence for wildlife as potential reservoirs of zoonotic viral pathogens in Rwanda: in particular, PREDICT detected several known and novel coronaviruses in bats – a family of viruses known for causing global pandemics like SARS and MERS, and the current COVID-19 pandemic.

As well, PREDICT Rwanda documented the "reverse" transmission of zoonotic pathogens from people to wildlife (gorillas).

Behavioral surveillance in people working in and living around Volcanoes National Park revealed a high level of daily contact with wildlife, and therefore the potential for virus sharing, or "spillover."

In a country with one of the most well-developed wildlife tourism operations on the continent, which brings people from all over the world into close contact with wildlife, the threat for bidirectional viral spillover is real.

It is therefore recommended that wildlife pathogen surveillance and characterization, comprehensive virus screening in febrile patients, and studies to better understand the ecological and behavioral drivers of virus spillover, be continued.

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

SPECIAL FEATURE

Satellite Enhanced Epidemic & Disease Network Model

PREDICT assisted scientists at UC Davis to develop an outbreak modeling tool that utilized human population and satellite-based geospatial data to simulate disease spread. The network model depicts human connectivity and mobility, commute rates, and roads connecting urban centers and rural settlements. In simulations, the tool was able to accurately predict the epidemic size and geographic spread of the 2009 H1N1 influenza A pandemic in Rwanda.

This work was published as an Emerging Disease Insights for USAID and is available electronically **https://bit.ly/3aVTJPi**

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GORILLA DOCTORS
Mountain Gorilla Veterinary Project & UC Davis Wildlife Health Center