

PREDICT UGANDA

One Health in action (2009-2020)

Elucidating sources and risk factors underlying wildlife zoonotic disease emergence and spread

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UGANDA

In Uganda, there are many opportunities for pathogens to emerge from wildlife that could pose a threat to human health. Uganda has a long-standing and well-established network of national parks, which provide protection for wildlife but are surrounded by dense human communities and intensive animal agriculture. Wildlife leave protected areas to forage in crops, and people live and enter parks for their livelihoods; this movement results in significant interaction and conflict among wildlife, livestock, and people. Uganda also supports a thriving wildlife ecotourism industry that brings both local and foreign people into daily contact with wildlife, including bats and primates, which present a zoonotic disease risk.

Since 2009, our team has been conducting field surveillance. building the One Health workforce, and strengthening systems for priority zoonotic viral diseases and other emerging threats in Uganda. During PREDICT, we put One Health into action through zoonotic disease surveillance in wildlife and people, including behavioral risk investigations at highrisk human-animal transmission interfaces. This work has directly supported the Uganda national

surveillance and laboratory systems, contributed to the development and implementation of the country's GHSA roadmap, and provided opportunities to strengthen mechanisms for responding to priority zoonotic diseases, such as avian influenza and hemorrhagic fevers (e.g. Ebola, Rift Valley Fever, and Marburg).

Working together, our team has supported the growth of Uganda's One Health workforce from the national to district level through field- and lab-based training, and engagement of national, university and non-governmental organization (NGO) staff and students in our surveillance and disease detection activities. PREDICT/Uganda trained wildlife veterinarians in the principles and practice of safe and effective wildlife surveillance, improved facilities, and acquired equipment for the safe, secure transport and storage of biological samples.

To better understand the threat posed to human health by viral pathogens circulating in wildlife, PREDICT/Uganda collected samples from wildlife in areas where wild animals had significant interactions with domestic animals and people. From 2009 – 2019, PREDICT/ Uganda sampled over 2,900 wild animals (>1,243 nonhuman primates, >900 bats, >760 rodents, and 60 other wildlife taxa); 80 camels; and over 400 febrile human patients. Sample processing and PCR testing was performed at Makerere University Walter Reed Project Laboratory and Uganda Virus Research Institute, with confirmatory sequencing performed at the University of California, Davis, and Columbia University. Animal and human samples were tested for up to 16 virus families / genera. In total, 59 viruses were detected, of which 20 were known and 39 were newly described viruses. Novel viruses belong to the Adeno-, Corona-, Herpes-, Paramyxo-, and Rhabdoviridae families.

PREDICT/Uganda also participated in several One Health disease investigations, including outbreaks of Ebolavirus (May 2011 and July 2012) and Yellow Fever (November 2010). The team contributed to behavioral risk and wildlife surveillance efforts to better understand the role that wildlife may have played in the outbreaks. In addition, these efforts enabled PREDICT staff to identify and relay the most effective strategies preparedness, for prevention, and response to communities and health professionals in order to protect people and wildlife from disease threats.

LOCAL PARTNERS

- Uganda Wildlife Authority
- Uganda Virus Research Institute
- Bwindi Community Hospital (Buhoma, Kanungu District)
- Makerere University Walter Reed Project
- Makerere University College of Veterinary Medicine
- Ministry of Agriculture, Animal Industry and Fisheries





DEVELOPED the One Health Workforce by training more than 70 people in Uganda.



OPERATIONALIZED One Health surveillance and sampled over 3.4K animals and people, to identify ways to help minimize the spillover of zoonotic disease threats from animals into human populations.

LABORATORY STRENGTHENING

Uganda Virus Research Institute
Makerere University Walter Reed Project





DETECTED 59 unique viruses in both animal and human populations.



"Bwindi is one of those places where the potential for viral pathogens to emerge from forest wildlife and spillover into both local and transiting populations is very high. Surveillance to date has shown this to be true, and with our PREDICT project, we now better understand how that spillover occurs and therefore how best to prevent it."



"PREDICT surveillance has shown that timely and accurate detection and reporting of infectious disease outbreaks help guide interventions to control epidemics. Now that Uganda has veterinarians with the capacity to safely capture and sample wildlife, and the training of laboratory staff in protocols for virus screening, the country has moved a major step ahead towards preventing, detecting, and responding to various health security risks and public health emergencies of international concern."

ACHIEVEMENTS

- Supported the Uganda National Task Force for Epidemic Preparedness and Response in its investigations of several disease outbreaks to better understand the role that wildlife may play in these events, and to learn lessons for the future
- Strengthened the National Task Force by encouraging a One Health approach to disease outbreak investigation, control, and prevention, resulting in incorporation of wildlife investigations into disease outbreak response planning
- Discovered a new coronavirus in a bat that is the most closely related to the one causing the current Middle Eastern Respiratory Syndrome (MERS CoV) outbreak, which sheds light on bats as the potential reservoir for emerging infectious diseases like MERS, SARS and the current COVID-19 pandemic

- Identified 39 novel viruses in non-human primates, bats, and rodents, including several new adenoviruses, herpesviruses, paramyxoviruses, rhabdoviruses and coronaviruses, as well as a known human coronavirus in a bat
- Developed a communication channel for sharing of surveillance test results with appropriate Government of Uganda ministries and departments

ONE HEALTH Surveillance



The overarching aim of PREDICT's One Health surveillance approach was to further build the evidence base for emerging infectious diseases and priority zoonoses at the human-wildlife interface. In Uganda, PREDICT focused its efforts in local communities living in close proximity to protected areas, working to better understand transmission pathways for viral spillover that could inform human disease prevention and control options.

Our surveillance work focused on the Southwestern districts of Kisoro, Rubanda, Kabale, Kanungu, and Rukungiri at sites where people and wildlife come into daily close contact through subsistence farming, wildlife tourism, and legal and illegal access to a highly biodiverse protected area, Bwindi Mgahinga Conservation Area. Bwindi and Mgahinga were fairly recently annexed by the Government of Uganda (in 1991) to serve as protected areas and the Batwa people who had previously lived and subsistence hunted in Bwindi were moved to communities surrounding the park. This part of Central – East Africa is among the most densely populated on the continent and is unique, as thousands of people come from around the world every year for the opportunity to view human-habituated mountain gorillas.



WILDLIFE & DOMESTIC ANIMAL SURVEILLANCE

To understand what viruses are circulating in wildlife that have the potential to infect humans, surveillance efforts focused on bats and rodents living in human dwellings and accessing agricultural grounds and crops in communities adjacent to Bwindi (primarily in Buhoma and Kisoro). From 2014-2019, a total of 572 wild bats (insectivorous and frugivorous) were safely and humanely captured in mist nets set up in neighborhoods, near roost sites, and across roads that people used to travel to, from and through Bwindi.

HUMAN SURVEILLANCE

People living in communities adjacent to Bwindi Impenetrable National Park who presented to Bwindi Community Hospital with fevers of unknown origin were included in this concurrent One Health surveillance effort. Bwindi Community Hospital is located in the town of Buhoma, at one of the main gateways to Bwindi, and as one of the best health centers in the area, is accessed by people coming from multiple villages and towns surrounding the park. Bwindi Community Hospital also serves park personnel





Numbers of individuals sampled by taxa

Likewise, 286 wild rodents were safely and humanely livetrapped in and around people's houses and croplands. In addition to intensive surveillance in the Bwindi area, there were specific opportunities to collect samples from wildlife utilizing highly urbanized environments (vervet monkeys in Entebbe), and from camels being utilized as pack animals, and thereby coming into very close contact with handlers and traders, in the Karamoja region (Moroto and Amudat Districts) of northeastern Uganda.

and their families, as well as the Batwa community. 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. From January 2017 to July 2018, 413 people were sampled and surveyed, including 107 adult men, 170 adult women, and 136 children.



VIRUS DETECTION

Samples from wildlife, humans, and camels were safely tested using consensus PCR (cPCR) to screen for priority zoonotic viral diseases and emerging threats such as for filoviruses (Ebola, Marburg), coronaviruses (SARS, MERS), paramyxoviruses, flaviviruses, and influenza viruses. Virus findings were confirmed through genome sequencing and interpreted to better understand the relationship of the

VIRUS TABLE

detected sequence to those from known animal and human pathogens. All tests were conducted at the Uganda Virus Research Institute (Entebbe, Uganda). Sequencing was conducted at the University of California, Davis and Columbia University. Test results were shared with the Government of Uganda through the appropriate ministries and departments and can be viewed at **www.data.predict.global**

			SAMPLING	IOTAL POSITIVE
VIRAL FAMILY	VIRUS	SPECIES	LOCATION	INDIVIDUALS
Coronavirus	Coronavirus 229E (Bat strain)	Noack's Roundleaf Bat, Geoffroy's Horseshoe Bat, Unidentified Hipposideros Bat	Rushaga, Kisoro	3
	Kenya bat coronavirus/ BtKY33/2006	Welwitsch's Myotis	Kisoro	1
	Kenya bat coronavirus/ BtKY66/65/63/60	Welwitsch's Myotis	Kisoro	1
	MERS-like coronavirus	Dusky Pipistrelle	Nkuringo	1
	PREDICT CoV-43	Geoffroy's Horseshoe Bat	Rushaga	7
	PREDICT CoV-65	Geoffroy's Horseshoe Bat	Rushaga	2
	PREDICT CoV-70	Geoffroy's Horseshoe Bat	Rushaga	1
	PREDICT CoV-46	African Giant Shrew	Kisoro	1
	PREDICT CoV-55	Unidentified Multimammate Mouse, Black Rat	Nkuringo, Nombe	2
	PREDICT CoV-71	Toad Mouse	Nombe	1
Paramyxovirus	PREDICT_PMV-71	Geoffrov's Horseshoe Bat	Rushaga	1
	PREDICT_PMV-68	Unidentified Crocidura Shrew, Stella Wood Mouse, Unidentified Swamp Rat	BINP	3
	PREDICT PMV-69	lackson's Soft-Furred Mouse	BINP	1
	PREDICT PMV-70	African Giant Shrew	Nombe	1
	PREDICT PMV-76	African Giant Shrew	Kisoro	1
Influenza virus	Influenza Ā	Human	Bwindi Community	1
			Hospital (Kisoro)	
Rhabdovirus	PREDICT RbdV-7	Lesser Short-Nosed Fruit Bat	Bunga	1
	PREDICT_RbdV-8	Unidentified Brush-Furred Rat	Kisoro	1
Picornavirus	Enterovirus J	Vervet Monkey, Olive Baboon	Mukono, Zirobwe, OFNP	32
	Rhinovirus C	Olive Baboon	ÕENP	1
Retrovirus	Baboon foamy virus	Olive Baboon	ÕENP	8
Adenovirus	PREDICT AdV-31	Black Rat	Nombe	1
	PREDICT AdV-32	Natal Multimammate Mouse	Kisoro	1
	PREDICT AdV-43	Unidentified Brush-Furred Rat	Nkuringo	2
	PREDICT AdV-47	Unidentified Swamp Rat	BINP	1
	PREDICT AdV-52	Stella Wood Mouse	BINP	1
Herpesvirus	Gorilla lymphocryptovirus 1	Mountain Gorilla	BIF	61
-	Baboon cytomegalovirus/ eye/Cll-163	Olive Baboon	QENP	4
	Cercopithecine herpesvirus 5	Vervet Monkey	Mukono	2
	Chlorocebus rhadinovirus 1	Vervet Monkey	Mukono, Nyamusingir	i6
	Gorilla rhadinovirus 1	Mountain Gorilla	BIF	1
	Green monkey lymphocrypto virus/marrow/Cll-040	Vervet Monkey	Mukono	3
	Green monkey lymphocrypto virus/marrow/Cll-051	Vervet Monkey	Nyamusingiri	1
	Macacine herpesvirus 4/Africa	Vervet Monkey	Mukono	4
	Macacine herpesvirus 4/Asia	Olive Baboon	QENP	1
	Panine herpesvirus 2	Mountain Gorilla	BIF	14
	Papiine herpesvirus 1	Vervet Monkey	Mukono	24
	PREDICT_HV-22	Vervet Monkey	Mukono	2
	PREDICT_HV-40	Vervet Monkey	Mukono	4
	PREDICT_HV-126	Mountain Gorilla	BIF	1
	PREDICT_HV-127	Mountain Gorilla	BIF	1
	PREDICT_HV-37	Unidentified Rhinolophus Bat	MGNP	2
	PREDICT_HV-43	Unidentified Tadarida Bat	QENP	1
	Rattus rhadinovirus 1	Black Rat	Kisoro, Nyanswiga	2
	PREDICT_HV-29	Unidentified Crocidura Shrew, lackson's Soft-Furred Mouse	Kisoro, BINP	3
	PREDICT HV-30	Big-Eared Swamp Rat	BINP	1
	PREDICT_HV-31	Unidentified Brus ¹ -Furred Rat, Unidentified Swamp Rat, Unidentified Soft-Furred Mouse	Kisoro	5
	PREDICT HV-32	Unidentified Brush-Furred Rat	Nkuringo Nombe	4
	PREDICT HV-33	Unidentified Brush-Furred Rat	Kisoro	3
		Unidentified Soft-Furred Mouse		-
	PREDICT HV-34	Stella Wood Mouse	BINP	2
		Unidentified Swamp Rat		-
	PREDICT HV-35	Big-Eared Swamp Rat.	BINP	2
		Unidentified Swamp Rat		_

PREDICT_HV-36 PREDICT_HV-38 PREDICT_HV-39	Unidentified Brush-Furred Rat African Giant Shrew, Black Rat Unidentified Brush-Furred Rat, Unidentified Swamp Rat	Nkuringo Kisoro Nkuringo, Nombe, Kisoro, BINP	2 2 8
PREDICT_HV-41	Unidentified Swamp Rat	BINP	1
PREDICT_HV-45	African Giant Shrew	Kisoro	1
PREDICT_HV-49	African Giant Shrew, Unidentified Crocidura Shrew, Natal Multimammate Mouse. Black Rat	Kisoro	5
PREDICT_HV-50	Unidentified Soft-Furred Mouse	Kisoro	1 252

BINP = Bwindi Impenetrable National Park; QENP = Queen Elizabeth National Park; BIF = Bwindi Impenetrable Forest; MGNP = Mgahinga Gorilla National Park

VIRUSES DETECTED IN HUMANS & WILDLIFE

Within PREDICT/Uganda from 2009 – 2019, we detected a total of 59 viruses belonging to a diverse array of viral families. A majority (39) of these viruses were novel discoveries, while the remaining 20 were known viruses. Rodents and shrews were host to 29 of these viruses, non-human primates hosted 18, bats hosted 11, and 1 virus was found in a human. The virus infecting a human was a known influenza virus (Influenza A) detected in a patient at the Bwindi Community Hospital in Kisoro. Subtyping to distinguish the H and N designations of this virus was recommended to ministry partners.

Total

The most viral diversity was found in the herpesvirus family, where we detected 33 unique viruses (21 novel and 12 known) predominately in non-human primates and rodents/ shrews. Coronavirus diversity was second highest, having discovered 10 unique viruses (7 novel, 3 known) infecting insect-eating bats and several rodents/shrews. A known coronavirus, Coronavirus 229E (bat strain), was detected in roundleaf and horseshoe bats within the *Hipposideros* and

Rhinolophus genera. This particular strain infects bats, and while there is a strain of a known alphacoronavirus that infects humans (Human Coronavirus 229E), which is known to cause respiratory illness in people, the bat-specific strain we detected is not considered a public health threat. Additionally, Coronavirus 229E (bat strain) was the only virus detected during both parts of the project in PRÉDICT-1 (2009-2014) and PREDICT-2 (2015-2019), but it was detected at different sites and in different bat species. One insect-eating bat, Geoffroy's Horseshoe Bat (Rhinolophus clivosus), was coinfected with two novel coronaviruses, PREDICT CoV-43 and PREDICT CoV-65. Neither of these two coronaviruses, nor any other novel coronaviruses discovered, are related to the novel coronavirus SARS CoV-2, the virus responsible for the current COVID-19 pandemic. All paramyxoviruses, rhabdoviruses, and adenoviruses discovered (12 total) were novel viruses predominately infecting rodents/shrews, as well as two bat species.

EPIDEMIOLOGIC & BEHAVIORAL RISK

Human surveillance conducted at Bwindi Community Hospital 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 January 2017 – July 2018, 413 patients participated in clinic-based surveillance and were administered behavioral

Self-Reported Human-Animal Contact in the Last Year

questionnaires. Fifteen (15) park staff also completed behavioral questionnaires. Interviews were structured to include questions on demographics, travel histories, knowledge of disease origins, medical history and contact with wild and domestic animals.





EDUCATION & DISEASE AWARENESS

Of patients surveyed who were older than 12, and who reported having had an illness in the past year, 93% (333/358) stated they did not know the cause of their illness or sickness. Less than 2% of respondents stated that their illness came from ingesting bad food or water, being bitten by mosquitos, or from contact with other sick people.

LIVELIHOODS

The predominant primary livelihood of all 428 participants was crop production (94% of females; 82% of males). The second most common livelihood was animal production, with 71% of females and 70% of males reporting raising animals. The most common animals raised were poultry, goats and sheep, and/or pigs. The remaining livelihoods included non-animal businesses and protected area workers.

SYMPTOMS

On average, patients presented to clinics after an average of 6.8 days with fever at the time of sampling. The most common symptoms in addition to fever were: headache (91%), dizziness (70%), loss of appetite (61%), abdominal pain (50%), malaise (50%), and chills (45%).

Patients were categorized in one of five categories for analysis: 1) fever with cough, 2) fever with vomiting/diarrhea, 3) fever with hemorrhaging, 4) fever with encephalitis, and 5) fever with headache. Males were 2.2 times more likely to have fever with cough than females. For the remaining symptom groups, there were no significant differences between genders. No symptom group varied significantly based on age groups of 0-10, 11-20, 21-40, 41-60, and 61+ years of age.

		# OF INDIVIDUALS
GENDER	FEMALE	242 (56.5%)
	MALE	186 (43.5%)
AGE	ADULT (≥18 YRS)	292 (68.2%)
GROUP	CHILD	136 (31.8%)
AGE	MEAN (SD)	26.3 (13.1)
	MEDIAN [MIN,MAX]	22.0 [11.0, 73.0]

REPORTED WILD ANIMAL CONTACT

Both males (91%) and females (96%) reported that most of their contact with wildlife was with rodents and shrews. Of all who reported contact with rodents, 82% reported finding rodents in and around their home, 77% found rodent feces in or around food, and 58% reported handling live rodents. Both males (42%) and females (24%) reported contact with wild birds, with most respondents reporting handling live wild birds. Few respondents (less than 5%) reported contact with other wildlife, including ungulates (e.g. duiker, buffalo), primates, carnivores, and pangolins.

REPORTED DOMESTIC ANIMAL CONTACT

Most respondents (94% of females and 95% of males) reported contact with poultry. Of those, 88% handled live poultry, 64% cooked or handled poultry raw, 60% found poultry feces around human food, and 66% reported poultry present in their home. Similarly, both males (90%) and females (94%) reported contact with goats and sheep, with 76% respondents reporting raising goats and sheep and 58% reporting cooking or handling raw goat or sheep meat. Both genders (male 70% and female, 65%) reported contact with cattle, with the most common contact types being handling live cattle (55%) or cooking or handling raw cattle meat (30%).

LIVELIHOOD-SPECIFIC ANIMAL CONTACT

Both wild and domestic animals were reported raiding food supplies or destroying crops by individuals engaged in animal and crop production. Rodents/shrews were most commonly reported, followed by goats and sheep, cattle, and birds. Individuals also reported raiding crops (21% of respondents) or livestock food supplies (14% of respondents) by nonhuman primates. Wild birds were also commonly reported to raid or destroy crops (75% of respondents) or animal food supplies (49% of respondents).

RISK COMMUNICATION

PREDICT utilized field surveillance activities as a platform for sensitizing local communities around its purpose. Community members and partner organization staff members learned about wildlife as the source of most human emerging infectious pathogens and the routes by which viral spillover occurs. In so doing, PREDICT created opportunities for community members and partners to engage in overall efforts through question and answer sessions. As well, PREDICT initiated local One Health outreach efforts in the greater Bwindi-Mgahinga Conservation Area, using tools developed by the project that convey easily understood, targeted messages to community members on how best to protect themselves and their families from bat-borne pathogens.

As the PREDICT Project came to a close in September 2019, our team held a symposium at Bwindi Community Hospital attended by over 100 participants representing the hospital, regional District Health and Veterinary Officers, Uganda VVildlife Authority – Bwindi park personnel, Village Health Teams, Bwindi Nursing School students, and community business leaders. During this meeting PREDICT provided information on project results to its government partners and the communities in which surveillance was conducted. This event was also an opportunity to share and distribute a practical tool, PREDICT's *Living Safely with Bats* behavior change and risk communication resource, which illustrates how communities can live safely with bats and protect themselves and their domestic animals from bat-borne diseases.



PRACTICAL IMPLICATIONS

In a country where multiple deadly viruses have spilled over from wildlife and caused outbreaks (e.g. Ebola, Marburg), and where other pandemic viruses (e.g. West Nile Virus, Zika Virus) with animal origins have been first detected, Uganda is a living laboratory for the threat of emerging infectious diseases resulting from humanwildlife contact.

PREDICT confirmed that viruses are circulating in wildlife in Uganda that are closely related to viruses known to cause human disease (e.g., a MERS-like coronavirus detected in a bat; see special feature on next page).

Intensive pathogen and behavioral surveillance in southwestern Uganda revealed a high-level of contact

among people and wildlife, and therefore the potential for virus sharing, or "spillover."

We detected serologic evidence for exposure to potentially lethal filoviruses (e.g. Ebola virus) in people living in the greater Bwindi Impenetrable Forest area and demonstrated that people who reported contact with forest wildlife (e.g. duiker) were more likely to exhibit antibody titers to filoviruses.

We recommend the continuation of wildlife pathogen surveillance and characterization, comprehensive virus screening in febrile human patients, and further studies to better understand the ecological and behavioral drivers of virus spillover.



IDENTIFICATION OF A NEW CORONAVIRUS CLOSELY RELATED TO MERS-CoV

During the first phase of the PREDICT project (2009-2014), we detected a new MERS-like coronavirus , PREDICT/PDF-2180 (GenBank accession number KX574227) from an insectivorous bat, *Pipistrellus cf hesperidus*, bat sampled in Kisoro District in 2013 within our concurrent One Health surveillance area. This virus is closely-related to the one that causes Middle Eastern Respiratory Syndrome (MERS). This finding provides further evidence for bats as the potential reservoir for emerging infectious diseases such as MERS, SARS, and COVID-19. To better understand the potential risk posed to human health by this virus, our Uganda team conducted in-depth molecular characterization of PREDICT/PDF-2180 and confirmed that the virus was not likely to pose a threat to human health. This important message was conveyed to government partners and Bwindi Community Hospital.

The open-access publication was published in the scientific journal *mBio*:

mbio.asm.org/content/8/2/e00373-17



EVIDENCE OF EXPOSURE TO EBOLAVIRUSES IN KISORO DISTRICT

During the first phase of PREDICT (2009-2014), PREDICT/Uganda implemented a separate but aligned research endeavor in partnership with Bwindi Community Hospital, implementing human behavioral surveillance and biological sampling in patients with fevers of unknown origin who reported contact with wildlife. Analyses of data resulting from this focused behavioral and serological survey that were completed during PREDICT-2 (2015-2019) demonstrated exposure to potentially lethal filoviruses (e.g. Ebola virus) in people living around Bwindi Impenetrable National Park. This special study also revealed that people who reported contact with forest wildlife (e.g. duiker – a common antelope species) were more likely to exhibit antibody titers to filoviruses, indicating that hunters and people who consume bushmeat are a particularly at-risk group for filovirus and Ebola virus spillover and spread.

Learn more at https://bit.ly/p2-uganda-filovirus

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







