PLOS | NEGLECTED TROPICAL DISEASES

REVIEW

What Factors Might Have Led to the Emergence of Ebola in West Africa?

Kathleen A. Alexander[1]*, Claire E. Sanderson[1], Madav Marathe[2,3], Bryan L. Lewis[3], Caitlin M. Rivers[3], Jeffrey Shaman[4], John M. Drake[5], Eric Lofgren[3], Virginia M. Dato[6], Marisa C. Eisenberg[7], Stephen Eubank[3]

1 Department of Fisheries and Wildlife Conservation, Virginia Tech, Blacksburg, Virginia, United States of America, **2** Department of Computer Science, Virginia Tech, Blacksburg, Virginia, United States of America, **3** Network Dynamics and Simulation Science Laboratory, Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, Virginia, United States of America, **4** Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York, United States of America, **5** Odum School of Ecology, University of Georgia, Athens, Georgia, United States of America, **6** Department of Biomedical Informatics, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, United States of America, **7** Departments of Epidemiology and Mathematics, University of Michigan, Ann Arbor, Michigan, United States of America

* kathyalx@vt.edu

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Abstract

An Ebola outbreak of unprecedented scope emerged in West Africa in December 2013 and presently continues unabated in the countries of Guinea, Sierra Leone, and Liberia. Ebola is not new to Africa, and outbreaks have been confirmed as far back as 1976. The current West African Ebola outbreak is the largest ever recorded and differs dramatically from prior outbreaks in its duration, number of people affected, and geographic extent. The emergence of this deadly disease in West Africa invites many questions, foremost among these: why now, and why in West Africa? Here, we review the sociological, ecological, and environmental drivers that might have influenced the emergence of Ebola in this region of Africa and its spread throughout the region. Containment of the West African Ebola outbreak is the most pressing, immediate need. A comprehensive assessment of the drivers of Ebola emergence and sustained human-to-human transmission is also needed in order to prepare other countries for importation or emergence of this disease. Such assessment includes identification of country-level protocols and interagency policies for outbreak detection and rapid response, increased understanding of cultural and traditional risk factors within and between nations, delivery of culturally embedded public health education, and regional coordination and collaboration, particularly with governments and health ministries throughout Africa. Public health education is also urgently needed in countries outside of Africa in order to ensure that risk is properly understood and public concerns do not escalate unnecessarily. To prevent future outbreaks, coordinated, multiscale, early warning systems should be developed that make full use of these integrated assessments, partner with local communities in high-risk areas, and provide clearly defined response recommendations specific to the needs of each community.

Introduction

On December 6, 2013, the world's largest Ebola epidemic began when a two-year-old in Guéckédou, Guinea, a small village bordering Sierra Leone and Liberia, became infected (Fig 1) [1,2]. This is the first documented Ebola outbreak outside Central Africa and is unique in its size, duration, and spatial extent. The circulating virus has been identified as the Zaire ebolavirus (EBOV), a strain previously found in only three Central African countries: the Democratic Republic of the Congo (DRC), Republic of the Congo, and Gabon (Fig 1) [3]. The public health impact of the current Ebola epidemic in West Africa has been far greater than case counts. Massive indirect effects on already-weakened public services have occurred, including significant crippling of the health sector, which has increased the impacts of other endemic diseases and the associated mortality [3]. Substantial economic loss and social disruption will have a sustained impact on the region that will far outlive the actual epidemic [4]. One paper, published in 2011, argued that Ebola would never become a significant public health threat in Africa [4]; clearly, the threat of Ebola has been underestimated. The emergence of Ebola in West Africa invites many questions—most of which remain unresolved—notably: why now, and why in West Africa? Advancing our understanding of this outbreak remains critical to present health care interventions as well as the prevention of further outbreaks. Here, we review the

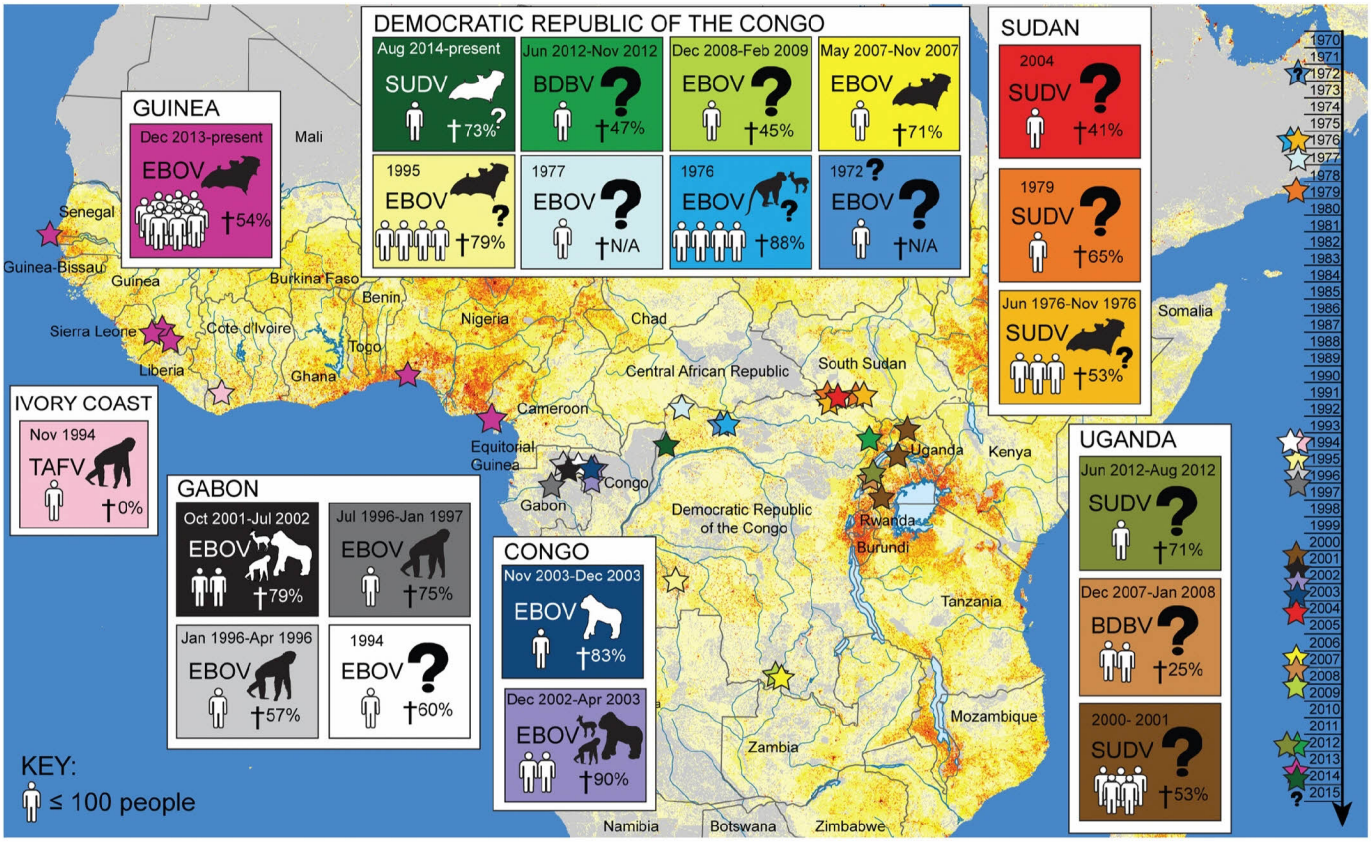

Fig 1. Map of Ebola outbreaks in Africa. The outbreak in West Africa is unprecedented in its scope and duration, occurring for the first time in urban centers. Historically, Ebola viral outbreaks (stars, timeline right) occurred sporadically, limited largely to Central African rural areas where the human population (grey to red gradient stippling [5,6]) has been low or more remote from areas of high population density. It is uncertain how frequent Ebola outbreaks will be in the future, given the identification of wildlife spillover potential in West Africa and the increasingly concentrated human populations in this region.

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sociological, ecological, and environmental drivers that could have influenced the emergence of EBOV in West Africa at this time and in this manner. Given these factors, we explore the lessons of this outbreak and evaluate how we might manage future threats from Ebola across the complex urban and rural landscapes that define modern Africa.

Ebola in Africa

Ebola hemorrhagic fever is an emerging zoonotic viral disease that historically has occurred in rural areas of Central Africa, with isolated cases identified elsewhere (Fig 1 and Table 1). The Ebola virus was first identified in humans in southern Sudan in 1976 [7], but likely occurred as early as 1972 in Tandala, DRC [8]. The virus causes severe morbidity and high mortality in humans and wildlife [9]. Humans typically are infected with Ebola either through contact with bodily fluids of infected animals or humans, or through consumption of bushmeat, caring for patients, or preparing the deceased for burial (Fig 2) [10]. EBOV can be found in a number of human secretions during the acute phase of infection, such as saliva, feces, semen, breast milk, tears, nasal blood, and skin [11]. Presently, there is no vaccine or other therapeutic interventions beyond supportive care, although promising pharmaceutical options are on the horizon, including vaccines [12].

Virus invasion in humans appears to occur through mucosal surfaces, breaks and abrasions in the skin, or parenteral introduction (reviewed in [18]). Route of exposure is important in determining the course of disease. During the 1976 outbreak in the DRC, the incubation period in humans exposed to EBOV through injection (in association with unsterilized needle reuse) was shorter than individuals exposed through known contacts (5–9 days, in respect of virus strains circulating in that outbreak [19]). Case fatality rates also differed by exposure route, with 100% mortality among those exposed through injection (85 out of 85) and 80% among cases with known contact (119 of 149). In laboratory studies of EBOV infection in nonhuman primates, the disease course was more rapid with exposure through intramuscular or intraperitoneal injection than through aerosol droplets [20]. Aerosol transmission has been identified only in laboratory settings [21] and is thought to be rare or absent in natural outbreaks [18]. Oral and conjunctival EBOV exposure was found to be extremely lethal in experimentally infected rhesus macaques [22]. Additionally, organs from laboratory-infected, nonhuman primates had extremely high infectivity titers (5.5–8.6 log10 pfu/g, [20]), indicating that exposure to high infectious doses might occur with consumption.

West African outbreak 2014

The World Health Organization (WHO) designated the West African outbreak as a Public Health Emergency of International Concern (PHEIC) on August 8, 2014 [23]. As of October 25, the WHO reported 10,141 cases and 4,922 deaths, making this ongoing outbreak several times larger than all previous Ebola outbreaks combined (Fig 3A) [24]. Even so, those numbers may be a drastic underestimate of the true case burden. In late August, the WHO estimates the true prevalence to be two to four times higher than the reported figures [25]. The outbreak is concentrated in the capitals of Guinea, Liberia, and Sierra Leone, although cases have occurred in nearly all regions of these countries.

Presently, there is little evidence of epidemic control in West Africa (Table 2) [26,27]. The recently developed model EbolaResponse provides a tool to estimate the potential increase in Ebola cases (available at http://dx.doi.org/10.15620/cdc.24900) [27]. It was predicted that if there were no significant changes made in outbreak management, the total number of Ebola cases could reach 21,000 in Liberia and Sierra Leone by the end of September 2014 [27]. This forecast included a correction for estimates of suspected under-reporting [27]. Despite this

Table 1. Ebola Outbreaks in Africa.

Date	Location of first case	Countries affected	Strain	Number of human cases	Number of human deaths	Mortality	Reservoir
Aug 2014–present	Equator Province, DRC	DRC	SUDV	67	49	73%	Possibly fruit bats
Dec 2013–present	Guéckédou, Guinea	Guinea, Liberia, Sierra Leone, Nigeria, Senegal	EBOV	5481*	2946	54%	Fruit bats
Jun 2012–Nov 2012	Province Orientale, DRC	DRC	BDBV	77	36	47%	Unknown, although bushmeat likely
Jun 2012–Aug 2012	Kibaale District, Uganda	Uganda	SUDV	24	17	71%	Unknown
Dec 2008–Feb 2009	Kasai-Occidental Province, DRC	DRC	EBOV	32	14	45%	Unknown
Dec 2007–Jan 2008	Bundibugyo District, Uganda	Uganda	BDBV	149	37	25%	Unknown
May 2007–Nov 2007	Kasai-Occidental Province, DRC	DRC	EBOV	264	187	71%	Fruit bats
Apr 2004–Aug 2004	Yambio county, Sudan	Sudan	SUDV	17	7	41%	Unknown
Nov 2003–Dec 2003	Mbono District, Congo	Congo	EBOV	35	29	83%	Gorilla
Dec 2002–Apr 2003	Mbono and Kéllé Districts, Congo	Congo	EBOV	143	128	90%	Possibly duiker, chimpanzee, and gorilla
Oct 2001–Jul 2002	Makokou and Mékouka, Gabon Border	Gabon, Congo	EBOV	122	96	79%	Possibly duiker, chimpanzee, and gorilla
2000–2001	Gulu, Masinsi, and Mbarara districts, Uganda	Uganda	SUDV	425	224	53%	Unknown
Jul 1996–Jan 1997	Booué, Gabon	Gabon	EBOV	60	45	75%	Chimpanzee
Oct 1996	Johannesburg, South Africa	South Africa	EBOV	2	1	N/A	Human travelling from Gabon
Jan 1996–Apr 1996	Mayibout, Gabon	Gabon	EBOV	37	21	57%	Chimpanzee
1995	Kikwit, DRC	DRC	EBOV	315	250	79%	Possibly fruit bats
Nov 1994	Taï National Park, Ivory Coast	Ivory Coast	TAFV	1	0	0%	Chimpanzee
1994	Mékouka, Gabon	Gabon	EBOV	52	31	60%	Gorilla
1979	Nzara and Maridi, Sudan	Sudan	SUDV	34	22	65%	Unknown
1977	Tandala, DRC	DRC	EBOV	1	1	N/A	Unknown
Aug 1976	Yambuku, DRC	DRC	EBOV	318	280	88%	Possibly antelope or monkey
Jun 1976–Nov 1976	Nzara and Maridi, Sudan	Sudan	SUDV	284	151	53%	Possibly fruit bats

Ebola outbreaks have been confirmed in Africa since 1976 [6]. Since then, four different strains of Ebola have emerged in Central and West Africa, from varying presumptive wildlife sources. These strains include Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Bundibugyo ebolavirus (BDBV) and Taï Forest ebolavirus (TAFV).

*Number of laboratory-confirmed cases only.

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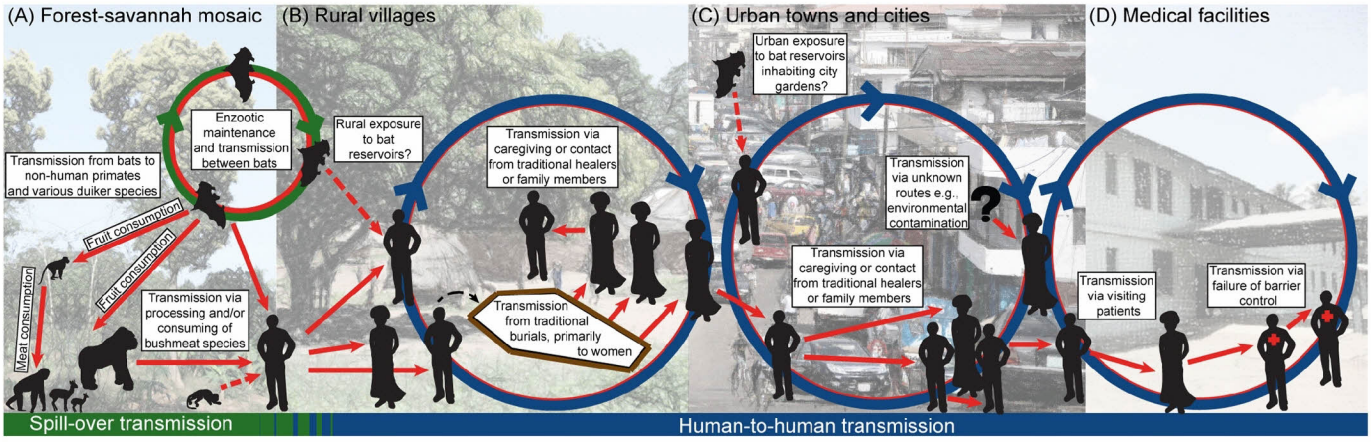

Fig 2. Schematic of virus spillover from wildlife and human-to-human transmission. Pathogen spillover to humans is typically associated with the use of bushmeat and direct contact with tissues and/or bodily fluids through handling and eating of infected animals (A), e.g., duiker, primates, or fruit bats [13]. Predation and consumption of a red colobus monkey by chimpanzees has also been linked to an outbreak of Ebola among chimpanzees and one researcher in Côte d'Ivoire [14]. Ingestion of fruit contaminated with Ebola-infected bat saliva or feces may be another mechanism by which bats might infect other involved wildlife species (e.g., duiker, nonhuman primates) or even humans. Human-to-human transmission has been associated with traditional burial practices, caregiving, or some other form of direct physical contact with infected individuals or bodily fluids [15]. Transmission dynamics in high-density urban centers (C) will differ importantly from rural villages (B), influencing outbreak progression and control efforts. Transmission in the hospital setting is largely associated with failures in infection control procedures and standard barrier precautions (D), many of which are related to inadequate staffing, infrastructure, and financing of health care systems [16,17].

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estimate being significantly less than the current reported number of cases [24], Ebola transmission is still widespread and intense in the West Africa region (Figs 3B and 4) [24,28]. EBOV infections have occurred beyond these core outbreak countries in Nigeria, Senegal, and Mali [24]. Model simulations using mobility and airline data indicate the threat of international dissemination beyond the Africa region through air travel is limited [29], despite secondary spread occurring in Spain and the United States [24]. Current intervention focus is on the rapid increase in treatment facilities and capacity to isolate infected patients in the affected countries in order to reduce Ebola transmission within the population [27,30].

Concerns of epidemic spread beyond Africa to places such as the US have occupied the public's attention and now have become important topics of concern and fear. A recent national survey found 39% of adult respondents believed there would be a large outbreak of Ebola within the US in the next 12 months [31]. Respondents with lower levels of education were more likely to express these views [24]. Unlike other viruses, such as influenza, that are airborne and can be transmitted through casual contact [32], Ebola requires direct physical contact with bodily fluids from a clinically ill person [15]. Accordingly, the only two cases of secondary transmission to occur in the US were associated with nursing staff and care of an Ebola patient [33]. Since these events, CDC guidelines and other safety protocols have been revised and strengthened [34]. More aggressive approaches, such as mandatory quarantine for returning medical personnel, have also been employed in some states [35], creating concerns that unnecessary fear and precaution may impact medical personnel and willingness to assist in the West African outbreak [36]. Public health education is urgently needed not only in West Africa but also within the US.

Full genome sequencing of EBOV isolates from the Sierra Leone outbreak region from May–August of 2014 ($n = 99$) [37] and previous molecular sequencing studies of a limited number of Guinean EBOV cases [10] provided similar results, suggesting that the West African outbreak arose from a single spillover event from a wildlife reservoir with subsequent sustained

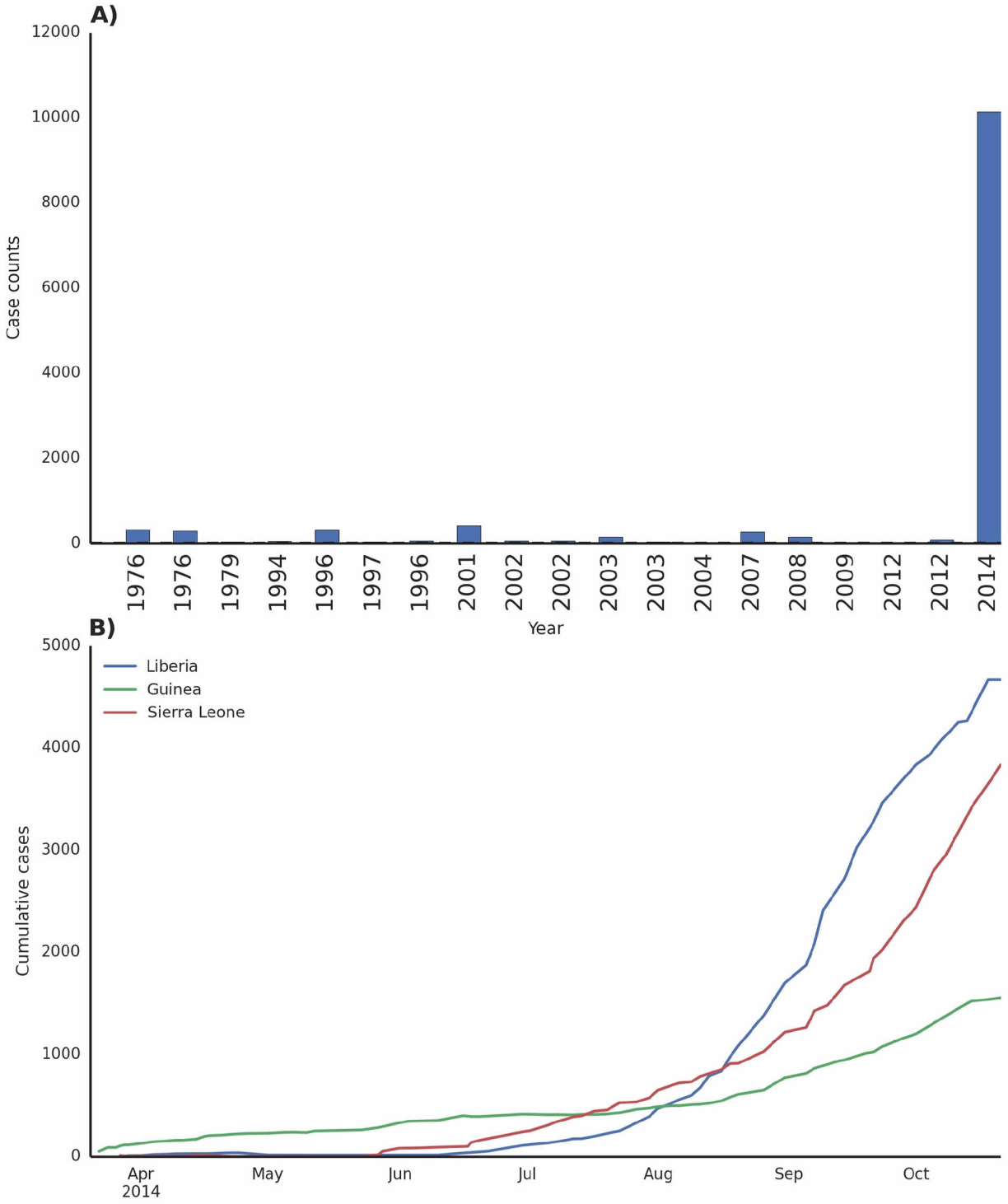

Fig 3. Case counts of historical Ebola outbreaks and the current outbreak in West Africa [24]. A) The 2014 West Africa outbreak eclipses all previous known outbreaks, with more cases and deaths than the other events combined. B) Cumulative case counts in Liberia, Guinea, and Sierra Leone demonstrate widespread transmission. Presently, Liberia is experiencing intense growth of the disease outbreak, with dozens of new cases each day.

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Table 2. Epidemiological characteristics of the 2014 West African Ebola outbreak.

Summary of Ebola outbreak characteristics in West Africa		
December–September 2014 [26]		
Term	**Definition**	**Current estimates**
Reproductive number (R_0):	*Number of healthy people one sick individual infects over the course of his/her illness.*	Guinea: 1.71
		Liberia: 1.83
		Sierra Leone: 2.02
Serial interval:	*Time between consecutive people falling ill in a chain of transmission.*	15.3 days
Incubation period:	*Amount of time passed between a person becoming exposed to Ebola and when they start to show symptoms of the disease.*	11.4 days
Doubling time:	*Time taken for the number of sick individuals to double.*	Guinea: 15.7 days
		Liberia: 23.6 days
		Sierra Leone: 30.2 days
Confirmed case fatality rate:	*Number of people who die of confirmed Ebola infection.*	Guinea: 70.7%
		Liberia: 72.3%
		Sierra Leone 69.0%
Unconfirmed case fatality rate:	*Number of people who die with suspected but not confirmed Ebola infection.*	Guinea: 13%
		Liberia: 58%
		Sierra Leone: 35%

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human-to-human transmission. However, important spatial and temporal limitations existed in sample collection in these studies. Accordingly, these conclusions can only be applied to the region of the outbreak area assessed. Additional studies will be necessary to fully understand EBOV transmission dynamics and the role of virus spillover from animal hosts.

Is the virus in the West African outbreak changing?

Phylogenetic analyses indicate that the virus strain in the current outbreak likely originated from Central Africa around 2004 [37]. In Sierra Leone, the outbreak is believed to have started from the introduction of two genetically different viruses from Guinea, where people were attending a funeral [37]. These two viruses diverged in Guinea in late April, before they were discovered in Sierra Leone a month later [37]. These sequencing efforts identified 396 genetic mutations that have occurred over time, including 50 nonsynonymous mutations since separation from the Central African lineage. During this current outbreak, the frequency of nucleotide substitution rates has been approximately two times higher than that observed across all previous Ebola outbreaks from which sequence data were available. Substitutions have been more commonly nonsynonymous [37], which change the amino acid sequence of the virus and could potentially be correlated with phenotypic changes that might influence outbreak dynamics and virus behavior. While more research is required to understand the effect of increased nonsynonymous mutation rates in the West Africa EBOV virus population, the sustained

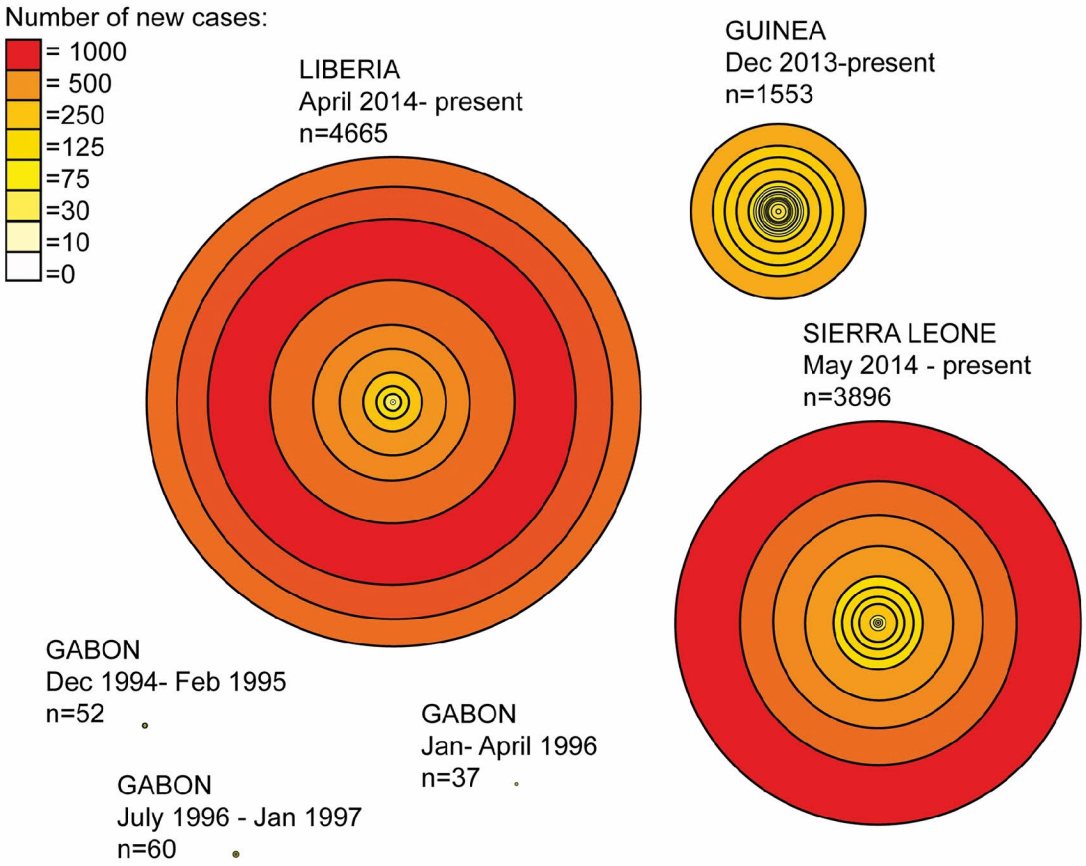

Fig 4. West Africa Ebola case counts at biweekly intervals. An assessment of EBOV outbreaks in which circles identify two-week intervals in outbreak progression, and distance between the circle lines is equivalent to the number of cases affected during that respective time period. The graphic highlights the important differences in outbreak duration and case counts not only between West Africa and Central Africa EBOV epidemics but also by country within the outbreak region in West Africa itself. Liberia clearly has had the largest number of cases over the shortest duration. This reflects, in part, the movement of the outbreak into the high-density urban center—the capital city Monrovia—and the intense growth of the outbreak from that point.

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nature of the outbreak increases the opportunity for further change in the virus, with uncertain consequences [37]. However, as yet, similarity in outbreak characteristics (including R_0, symptoms, incubation time, serial time) between the West Africa 2014 outbreak and previous Ebola outbreaks suggests that there has not been any significant change in the virus affecting transmissibility (Table 2) [26]. Rather, outbreak progression appears to be more strongly influenced by the urban setting of the outbreak and other socioeconomic features.

The Democratic Republic of Congo 2014

A second outbreak of Ebola was discovered in the rural Boende region of the DRC in August 2014 (Figs 1 and 5). The index case was identified as a pregnant woman who handled bushmeat. Subsequent infections in the community stemmed from contact with the woman's body during funeral rituals [38]. Phylogenetic analysis confirmed it to be a different strain, unrelated to the 2014 West African outbreak, indicating that a separate zoonotic introduction was responsible for viral emergence into the DRC population [39]. This virus strain is most closely related to virus isolated from the 1995 Ebola outbreak that occurred in Kikwit, DRC. As of October 21, 2014, the outbreak had grown to 67 cases and 49 deaths [38].

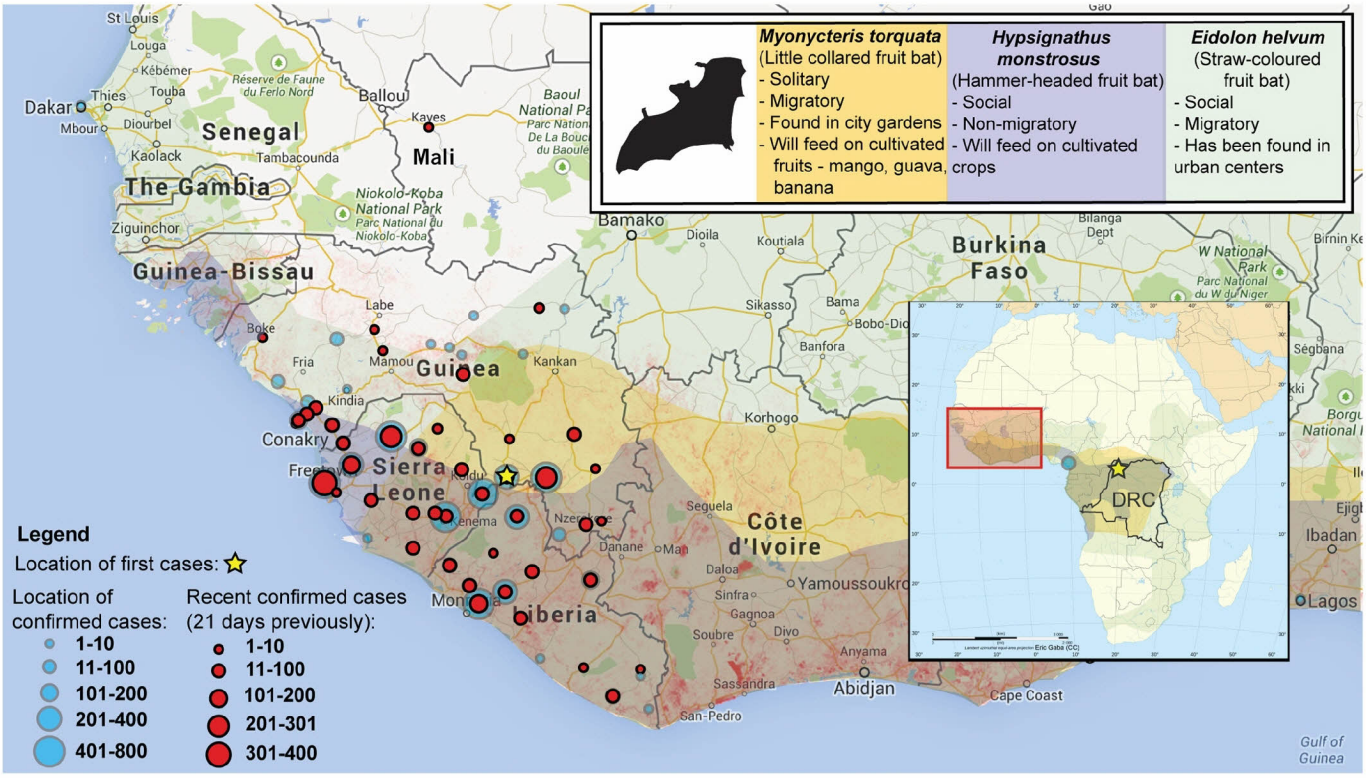

Fig 5. Range of bat species suspected of being reservoirs of Ebola, human population density, and Ebola case counts by location in West Africa. The range of putative EBOV reservoir species the little collared fruit bat (yellow), the hammer-headed fruit bat (blue), and the straw-coloured fruit bat (green) are thought to be associated with previous Central African EBOV outbreaks [40–42]. Guéckédou, Guinea, was the first affected area in December of 2013 (star) [12] with spread to other regions (blue—location of confirmed, red—recent confirmed cases as of October 20, 2014 [43]. The outbreak now involves Sierra Leone and Liberia. Limited spread, in Nigeria and Senegal (only one case), related to travel of infected persons has been identified. A separate Ebola outbreak in the DRC was reported on August 25, 2014 (map inset) [44]. Human-mediated loss of forest resources (2000–2012, red stippling) has been dramatic in the region [45]. In addition to bushmeat-associated exposure, human-mediated environmental change in the region could increase human contact with potentially infected bat species in both the urban and rural environment.

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Pathogen Spillover

Spillover of EBOV from the wildlife reservoir to human populations appears to be a complex process involving a number of coupled networks and seasonal drivers (Fig 2) [46], linking the human host to virus reservoirs. Several bat species are considered to be putative EBOV reservoirs, three of which have been a focus of attention with respect to the current West African outbreak: the hammer-headed fruit bat (*Hypsignathus monstrosus*), the little collared fruit bat (*Myonycteris torquata*), and the straw-coloured fruit bat (*Eidolon helvum*) [41,42]. Only frugivorous and insectivorous bat species have shown virus replication and developed high circulating virus titers without showing EBOV-associated illness [47]. Virus found in lung tissues and feces indicates that respiratory, oral, and fecal transmission pathways may all be possible exposure routes to susceptible hosts. Outbreak range overlap is identified in a number of bat species in which EBOV antibodies have previously been found (Fig 5). Some bat species, such as the straw-coloured fruit bat, the largest ranging bats species in Africa, have the ability to migrate long distances (up to 2,500 km) [48]. Thus, movement of EBOV through bat colonies from Central Africa into West Africa would be possible. Alternatively, the virus may have been in the reservoir host for some time, but conditions for spillover did not occur previously.

EBOV transmission to wildlife species (e.g., duiker, nonhuman primates) is thought to occur with ingestion of fruit that has been contaminated with infected fruit bat saliva or feces [41]. Chimpanzees, however, are different and in addition to consuming fruit, will actively engage in predation of other wildlife and nonhuman primates, hunting cooperatively and sharing meat among their social group, a behavior rarely observed in other nonhuman primates, with the exception of baboons [49]. In addition, only chimpanzees will carry meat away from the site of predation—in some instances, more than a kilometer. Scavenging of meat from carcasses is, however, rarely identified among any nonhuman primate species. Chimpanzee hunting has previously been linked to Ebola emergence in Côte d'Ivoire, where the hunting and shared consumption of a red colobus monkey was associated with a large outbreak of Ebola among chimpanzees (Fig 2) [14]. This was the first record of "bushmeat" consumption causing an Ebola outbreak in a nonhuman primate population. A large-scale survey of nonhuman primates across Central Africa only found significant serologic evidence of exposure among chimpanzees (12.9%), suggesting that non-lethal infections do occur in nonhuman primates [50]. Seropositive chimpanzees were found broadly throughout forested regions of Central Africa, identifying Ebola viral circulation in areas where human infections have not yet been identified (e.g., Cameroon [50]). Serosurveillance studies among humans in Central Africa have also identified seropositive individuals even in the absence of a history of Ebola infection or residence in an area where an Ebola outbreak occurred [51]. Understanding spillover in humans continues to be a challenging issue, given the relative infrequency of these events. Important similarities exist in both the physiology and behavior of chimpanzees and humans. Focused research on chimpanzees might provide important insight into Ebola spillover pathways arising from hunting and consumption of bushmeat.

What is the role of nonhuman primates in virus circulation?

Ebola is a rapidly fatal disease for nonhuman primates [52]. Although a potential source of infection for humans through consumption of dead apes, nonhuman primates are not considered to be a reservoir host or a host species able to maintain sustained viral transmission independent of contact with the reservoir host [52]. Indeed, outbreak mortality in chimpanzees and gorillas has been extreme with some outbreaks, pushing these species closer to extinction [9]. The 2002–2003 epidemic of Ebola in gorillas in the Lossi Sanctuary in northwest Republic of Congo killed 90%–95% of the population, an estimated 5,000 animals [53].

Seasonal triggers of Ebola outbreaks

Meteorological factors have been associated with a variety of infectious diseases and can have complex influence over contact networks and disease transmission pathways [54]. This is particularly true when wildlife reservoirs are involved in pathogen spillover to other wildlife species and humans. Local and regional weather patterns act as a strong determinant of floral characteristics and surface water attributes within a given landscape. The nature and distribution of these resources can dynamically influence animal behavior, as well as species distribution, species fitness, migration patterns, and population density. These attendant effects can have a profound impact on contact probabilities between susceptible and infected hosts within and between species, as well as the potential for pathogen transmission and spillover to humans [55]. Additionally, contact probabilities can be altered by deforestation and land-use change, which may compound the impact of meteorological phenomena and further cluster susceptible and infected hosts around more limited resources.

There has been little analysis of the meteorological and hydrologic conditions associated with Ebola outbreaks. Study of these processes has been hampered by the limited availability of

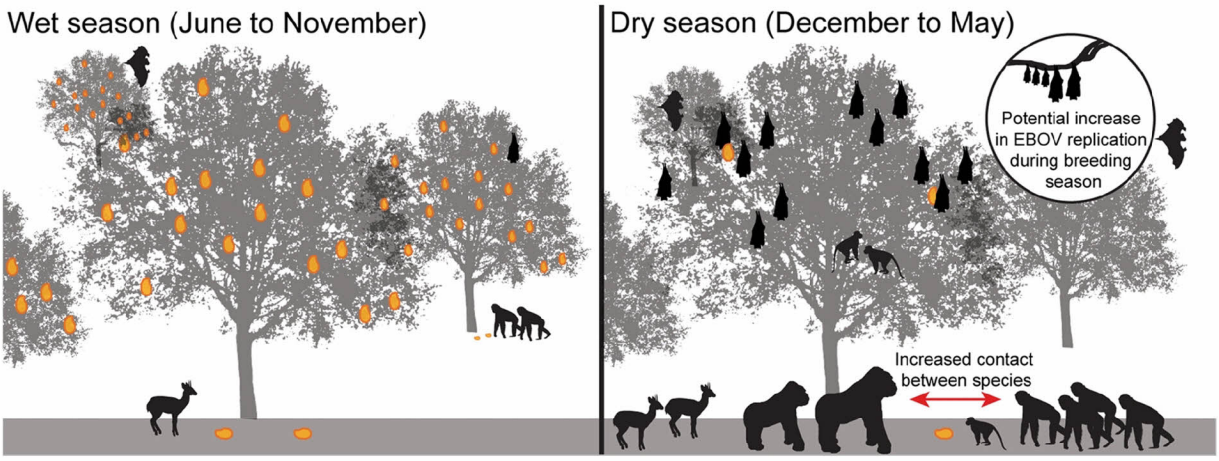

Fig 6. Seasonal factors may influences forage and wildlife distributions, potentially increasing their contact with Ebola reservoirs. Ebola outbreaks appear to coincide with seasonal factors, which can influence forage availability and spatial distribution across the landscape, potentially increasing contact between wildlife species and EBOV transmission potential. Fighting and breeding among bat species during these periods is thought to potentially influence viral load and EBOV transmission within and between bat species.

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meteorological station observations in Central Africa. A few studies have attempted to circumvent this issue through use of satellite estimates of land surface greenness. In this fashion, Pinzon et al. (2004) examined eight Ebola outbreaks during 1994–2002 and found an association with drier-than-normal conditions at the end of the rainy season. Certainly, hydrologic changes could influence forest fruit production and other resources. Foraging behavior in frugivorous species (e.g., fruit bats, duikers, and nonhuman primates) can be strongly influenced by seasonally driven temporal and spatial clustering of scarce fruit resources [56], potentially concentrating reservoir and susceptible host species in these areas of increased foraging opportunity (Fig 6). A recent study identifies the potential zoonotic transmission niche as a region that covers more than 22 countries in Central and West Africa. These areas are defined by characteristic vegetation, elevation, temperature, evapotranspiration, and range of suspected bat reservoirs [57].

Human-mediated landscape alteration—Increased contact with EBOV reservoirs?

In the outbreak zone, human-mediated environmental change has been significant, potentially contributing to the emergence of EBOV. The Guinean forest surrounding the outbreak areas is considered a major biodiversity hotspot, containing an estimated one-quarter of all African mammalian fauna [58]. Human encroachment into these areas has been dramatic, with cumulative forest loss estimated to be between 83%–86% (Fig 5) [59]. The landscape is now dominated by forest-agricultural mosaics [58]. These environmental changes in the outbreak region may provide the opportunity for direct exposure to infected bats, potentially creating transmission pathways that do not rely on exposure to bushmeat. For example, the little collared fruit bat can be found in forest/grassland mosaics, an increasing feature of the landscape, and has been identified feeding on guavas and mangoes [60], as well as occurring in more urban areas such as city gardens [61]. Straw-coloured bats have also been identified in human-modified environments, including city parks [62]. The hammer-headed fruit bat can be found in a wide range of habitats, including agricultural areas, where they have been recorded feeding on cultivated crops [63]. The two-year-old child who is the index case in the West African outbreak is

assumed to have been exposed by eating bushmeat [10]. However, the child could well have been exposed to bat-contaminated fruit or other bat excretions within the home environment where EBOV-infected bats may occur [41]—a more likely exposure route than eating bushmeat if, indeed, the two-year-old was the first case. It will be important to determine whether Ebola spillover can occur independently of bushmeat utilization and exposure.

Social Conditions Enabling and Enhancing Human-to-Human Transmission

War, population growth, poverty, and poor health infrastructure, among other social conditions in the outbreak region, have likely contributed to the unprecedented expanse, duration, and size of the EBOV epidemic in West Africa (Table 3). In this region of Africa, population growth has been dramatic, with population densities (people/km^2) increasing by 223%, 178%, and 275% in Guinea (1960–2012), Sierra Leone, and Liberia, respectively (1961–2013, Fig 7A) [64]. Rural-to-urban migration and growth in the affected countries has significantly increased the proportion of people living in urban environments, where EBOV outbreaks have focused in West Africa. The proportion of the population that is now urbanized has increased significantly in Guinea (248%, 1960–2013), Sierra Leone, and Liberia (130% and 163% respectively, 1960–2013, Fig 7B) [64].

Human mobility

A complex suite of sociological and economic factors influence human movement across the landscape and can have critical impacts on outbreak dynamics and the spatial spread of infectious disease [65]. In West Africa, human movement is considered a particular characteristic of the region [66], with migration rates exceeding movement in the rest of the world by more than 7-fold [67]. An estimated 11% of West African people live outside their country of birth, with between 30%–40% of people residing outside their district or village of birth [68]. In Liberia, for example, 54% of the population over the age of 14 are identified as being internally

Table 3. Socioeconomic and environmental factors may have influenced Ebola emergence in Guinea, Liberia, and Sierra Leone [64].

	Country	Guinea	Liberia	Sierra Leone
Environmental features	Country size	94,926 sq miles (245,857 km^2)	43,000 sq miles (111,370 km^2)	27,699 sq miles (71,740 km^2)
	Crop production index increase (2004–2006 = 100) (1961–2012)	246%	118%	388%
	Livestock production index increase (2004–2006 = 100) (1961–2012)	346%	305%	328%
Human resources and infrastructure	Number of physicians (per 1,000 people in 2010)	0.1	0.01	0.02
	Improved sanitation (Total, Rural, Urban)	19%, 11%, 33%	17%, 6%, 28%	13%, 7%, 23%
	Improved water source (% of population without access in 2012)	25%	25%	40%
Population features	Urban population increase (% of population (1960–2013)	223% increase (1960–2012)	275% increase (1961–2013)	178% increase (1961–2013)
	Historical civil unrest	Yes	Yes	Yes
	Literacy (% of people age 15 and above)	25% in 2010	43% in 2008	44% in 2012
Cultural and behavioral features	Use of traditional healers	High	High	High
	Use of traditional burial practices	High	High	High
	Bushmeat consumption	High	High	High

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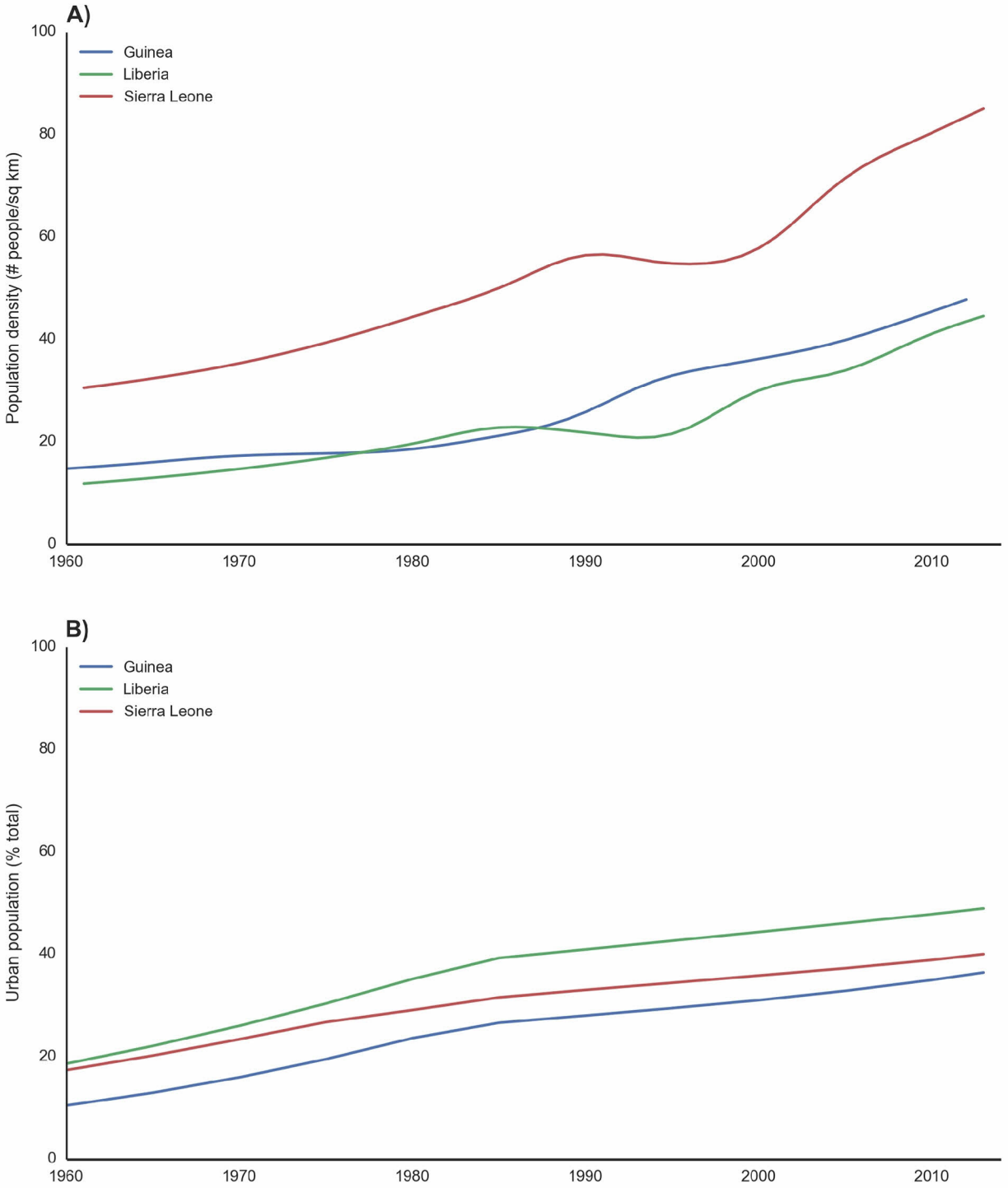

Fig 7. Increases in population density and the proportion living in urban environments in Guinea, Liberia, and Sierra Leone since the 1960s. A) Population density in the outbreak region has increased dramatically over the last 40 years [64]. Increases in human density can have a critical influence on contact networks and human-to-human transmission potential and environmental degradation. Increasing need for natural resources can potentially increase contact rates with wildlife (e.g., timber). B) Urbanization is an important factor influencing infrastructural needs, resources, and population density, factors that can influence contact networks, outbreak dynamics, and intervention success. This is particularly true in poorer countries, where rapidly progressing disease outbreaks in urban environments outstrip weak public health resources. Liberia has experienced the greatest increase in urban population, with an estimated 253% increase since 1961.

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displaced [69]. Large-scale population movements in the region, both within and between countries, have been driven by decades of conflict and the search for improved socioeconomic conditions and opportunities, identifying an important part of regional livelihood strategies for the poor [68]. As such, present-day population mobility in West Africa has been an important contributing factor to the explosive nature of the West African Ebola outbreak.

The location and nature of the index case and spillover event has also been important to the rapid spread of the epidemic. In this case, the index cluster of infections occurred in Guéckédou, Guinea, a small village bordering Sierra Leone and Liberia near major road networks [1,2]. Infected individuals moved rapidly from the originally infected village into other locations, eventually leading to human introduction of EBOV into major urban centers, such as the capital city of Liberia, Monrovia (mid-June 2014) [70]. Regional expansion of the outbreak to Senegal and Nigeria was associated with travel from affected regions. Fear of rapid Ebola spread across the continent and globe has precipitated border controls on movement to and from the affected countries [71]. Border controls themselves, however, can have important negative impacts on the outbreak, preventing movement of urgently needed supplies and resources, prompting the United Nations Security Council to call for an end to the isolation of affected countries [71].

Decades of civil unrest

From 1989 to 2004, sustained armed conflict raged in West Africa, moving across borders among Liberia, Sierra Leone, Guinea, and Côte d'Ivoire. Violence, looting, and pillaging became an economic opportunity for impoverished people, and a large mercenary force developed in the region [72]. Mass refugee movements and resettlement camps created a large group of displaced and vulnerable people, with the associated environmental impacts that persist today [73]. These regional environmental and societal disturbances have impacted infrastructure, governance, social cohesion, and the mental and physical health and livelihoods of people in the region [74,75]. These effects have also severely undermined societal resiliency as well as public health infrastructure and service delivery in the region [75,76].

Behavioral and cultural practices

Consideration of behavior and culture in disease transmission is critical to control and understanding transmission dynamics [77]. Cultural diversity shapes African nations between and within countries and can have a profound influence on social cohesion and communication, particularly during times of disturbance. For example, Liberia has at least 16 major ethnic and cultural groups, each described by a specific language and associated dialects, religion, traditions, and customs [78]. EBOV, because of its nature of transmission, is particularly influenced by cultural and behavioral practices that occur at the household and community levels and within a hospital setting (patient care, family involvement and role, health-seeking behaviors and responses). Consequently, there is no one "community," and the cultural diversity that defines the region will need to be considered in local disease emergence prevention as well as in the public health response.

Bushmeat consumption

Bushmeat utilization has been identified as the primary mechanism of EBOV spillover from wildlife reservoirs to humans. Rapid human migration to urban centers has placed increased pressure on the region for food production [58], including access to bushmeat, a preferred protein source [79]. In Liberia, timber extraction, opening of road networks, and influx of worker settlements has been linked to unprecedented increases in bushmeat extraction from forested

regions [80]. Bushmeat in Liberia is a critical source of protein, estimated to account for three-quarters of the country's meat use [81]. In Brazaville, Republic of Congo, 88% of households interviewed reported consuming bushmeat (n = 1,050), preferentially mammals (artiodactyls [48.3%], rodents [28.3%], and primates [13.0%]) [82]. Bushmeat has become an important commercial commodity, trafficked illegally both domestically and internationally, potentially providing a mechanism for pathogen spread. Indeed, it has been estimated that approximately 5 tons of bushmeat are illegally imported into Europe each week [83], and it is a common form of contraband moved within and between African nations [84]. While the Ebola virus is susceptible to a variety of disinfectants and can be inactivated by cooking (60°C for 60 minutes) or boiling for five minutes [85], the virus can survive over three weeks at low temperatures in the absence of disinfection or inactivation [86]. This is consistent with epidemiologic data, which identified disease in game hunters [87–89], with none documented in individuals who ate the game after cooking [87]. Wildlife biltong, a dried-meat delicacy that is widely consumed in Africa and abroad, may pose special challenges [90], given that the virus can survive over 50 days when dried and kept at 4°C [86]. At present there have been no confirmed cases of Ebola related to the consumption of dried or smoked meat. However, there is still the concern that movement of biltong could increase the infection risk of wildlife products well beyond the point of animal slaughter to distant markets, given virus survival potential. Cultural practices can also differ importantly as to what wildlife species are used, obtained, processed, and consumed, potentially influencing Ebola transmission risk [77].

Burial practices

Traditional burial practices, involving washing and touching of the deceased, have been linked to 60% of Ebola cases in Guinea [91]. Caregiving, primarily by women, has also been associated with outbreaks, presumably explaining the relatively high rate of infection in women (67% of affected individuals) in the 2000–2001 Ugandan outbreak [92]. When a traditional healer fell ill with Ebola in Uganda, many individuals from the community came to care for her, and when she died, they took part in her burial [92]. The infected individuals were all women. Spread of the present outbreak into Sierra Leone was also associated with infection and death of a traditional healer and the women who had participated in her funeral [37]. It is important to note that burial practices can be divergent even within a nation, giving rise to the need to consider ethnic diversity and cultural differences within and between villages, towns, nations, and regions and their influence on funeral practices and pathogen transmission dynamics. There is a need to identify more refined data on these activities so that appropriate regionally and culturally specific public health practices can be developed. These data will also aid efforts to model epidemic dynamics; as funerals are an important feature of transmission, the nature of them will define epidemic spread.

Traditional medicine and cures

Traditional medicine is defined as the total knowledge base, skills, and associated practices that arise from theories, beliefs, and experiences identified by different cultures and used in the maintenance of health. Traditional medicine constitutes the world's oldest health care, and it has involved the development of culturally and geographically specific techniques for preventing illnesses and diagnosing and treating individuals and communities for centuries. While modern health care based on Western medicine is now considered the norm in many countries, much of Western Africa still relies heavily on traditional practices. Indeed, in countries surrounding the outbreak zone, such as Cote d'Ivoire and Ghana, 70% of the population depend solely on traditional medicine, while in Burkina Faso and the DRC, this figure increases

to 80% of the population [93]. While traditional medicine can have a positive role in health care, ethnomedical beliefs can also have important impacts on health-seeking behavior, health outcomes, and pathogen transmission pathways.

Individuals often look to traditional healers and family members for advice and care despite inexperience of the person providing information [94]. Traditional healers may have positions of influence within the community and, therefore, command a level of trust, and can also have a significant influence on health-seeking behavior and uptake of health messages, factors that can directly affect outbreak dynamics. Sick individuals have often opted to listen to traditional healers and rumors about potential "cures," for example the use of saltwater baths and drinks that have led to recent deaths in Nigeria [95]. Drinking bleach was also considered a way to rid oneself of Ebola in the Ugandan outbreak of 2000–2001 [96]. In the 2005 Ebola outbreak in the Congo, traditional healers declared that cursed "dishonest hunters" caused the outbreak, and many believed this to be true [97]. False information of this sort can significantly affect outbreak dynamics and increase the length and severity of epidemics. Encouragingly, the head of traditional healers in one district of Sierra Leone has recently stopped treating patients, acknowledging that he knows very little about the virus, and called on other healers to suspend healing activities until they are given adequate training [98]. Training traditional healers in infection control and delivery of public health messages might be an important mechanism for the dissemination of information to local communities and reduction in Ebola transmission risk.

Fear and obstruction of health interventions

Immense fear and anxiety exists toward modern health care providers in Ebola outbreak countries. This fear has stopped many individuals from seeking health care, causing them to instead hide from authorities and revert to traditional healers or family members for care [91]. Sick individuals already admitted to health care facilities have also fled, fearing they will only die in the hospital environment [91]. For example, in the Ugandan outbreak, people feared that once they went to hospital they would never see their families again [92]. In a rural setting, these influences will be important, but in high-density communities, they can be catastrophic in their effect on outbreak dynamics and control efforts. While health care and aid workers have the very best of intentions, the nature and severity of the virus means that quick action must be taken, resulting in the breakdown of communication between patients, relatives, and workers, and the inability of traditional practices to take place, propagating more fear and distrust between the parties. This outcome stems in large part from a lack of understanding and familiarity with Western medicine and practices [94], whereas community values often prioritize traditional practices and consultation and see both as a critical step in any community process engendering trust. For example, with the immediate need to disinfect and dispose of infected corpses, health care workers carried out burials before notifying families [92]. In 1995, during the Kikwit epidemic, all deceased individuals were buried in individual or common graves by the Red Cross staff. The body of one individual, however, was forcibly taken from the hospital to the family's home to have a traditional burial [99]. The removal of this body led to another (and the final) surge of Ebola infections in Kikwit [99].

Fear is not limited to community members, but is also common among health care workers [96]. These concerns are not unwarranted, as hospital staff are at an increased risk of exposure [100]. Health care worker infection can be catastrophic, particularly where large populations are served by an inadequate public health sector. By September 2014, in the West African outbreak, 10% of the deceased were believed to be health care workers [26]. In the Kikwit outbreak in 1995, 25% of Ebola cases were health care workers, and many left their jobs out of fear of

contracting the disease [96,101]. Understaffing of hospitals involved in Ebola outbreaks has led to staff working longer and harder, resulting in exhaustion and an increased potential for deadly mistakes.

Stigmatization and implications to outbreak containment efforts

Health stigmas can influence the behavior of both the infected and the uninfected during an epidemic, introducing barriers to outbreak management and potentially influencing pathogen transmission and spread, as well as disrupting social cohesion. The AIDS pandemic provided important insight into the critical impacts health-related stigma and social hostility can have on epidemic control measures, highlighting the absolute need to consider these elements in public health strategy development and response [102]. Ebola has perhaps provided a more extreme example. Health care workers, critical to outbreak management, have been harshly stigmatized during Ebola outbreaks, rejected by their communities and families, and even stoned by community members, as they were believed to act as a reservoir for the virus [99]. These same beliefs and stigmas have impacted health-seeking behavior, with the fear of contracting Ebola from health care workers influencing the decision to seek medical help [103]. Fear of stigmatization can influence disease reporting, with victims and their families failing to notify authorities of possible infection because of the potential negative response of their neighbors and community [104]. Ebola survivors can also be heavily stigmatized—many survivors are rejected by their communities, have their belongings burned, and are not allowed to share common amenities [92]. Data from the 2001 Uganda outbreak suggest female survivors experienced more stigmatization than male survivors [92]. Stigmatization can reach beyond the immediate family, as for example in Uganda, where relatives of survivors and the deceased were also stigmatized once the names were publicly released [96]. Fear of stigmatization is not necessarily limited to the level of the individual, household, or community, but may also extend to the level of country governance in which concerns over international response may influence reporting of health information [105]. Health-related stigma has been a prominent feature of the outbreak in West Africa [104] and likely a contributor to the difficulties identified in containing the epidemic.

Health education is one of the keys to combating many issues surrounding Ebola outbreaks, including trust of health officials, the use of non-traditional burial practices, and the acceptance of survivors, relatives of the deceased, and health care workers back into their communities. Health education was seen as one of the major factors in stopping the DRC Ebola outbreak in 1995 [101], and along with contact-tracing and quarantine in the Congo (1995) and Uganda (2000) outbreaks, health education was believed to decrease the effective reproductive rate of Ebola and reduce the final epidemic size by a factor of 2 [106]. However, as important as it is to develop and share health messages, the messages must engage the culture and traditions of the target group or risk having no effect or, worse, a negative effect.

Ebola Forecasting, Detection Control, Education, and Future Needs

Containment of the West African Ebola outbreak is the most pressing, immediate need. This effort will require mobilization of many additional resources, including medical personnel, educators' supplies, food, water, and other essential needs. Additional issues need to be addressed to prepare other countries for the possibility of Ebola importation or emergence. Below, we highlight example recommendations that might support enhanced country-level preparedness in Africa and elsewhere, while recognizing that many of these recommendations may be very difficult to implement in the West African countries currently combatting Ebola.

1. **Partnerships and coordinated outbreak response:** Coordinated development of communication strategies and surveillance partnerships across the region will be needed. Governments outside the outbreak region will need to be actively included and assisted as needed to develop national detection and response strategies and protocols. Regional meetings of Health Ministers (e.g., 2nd Extra Ordinary Ministers of Health Meeting on Ebola Virus Disease in Victoria Falls, Zimbabwe, September 4–5, 2014) provide important venues for bringing scientists and policymakers together to ensure that frontline countries have access to the resources they need to manage potential spread and outbreak response. Developing sustained partnerships across Africa and the international community will be critical for our ability to contain this and future epidemics.

2. **Outbreak response in resource-poor settings:** Information collection and communication will still be a challenge in resource-poor settings, and specific strategies will need to be developed to allow rapid identification and response within the context and constraints identified in the local environment. Integrated approaches involving both human and animal health must be developed that engage the research, law enforcement, and policy environments within these local settings.

3. **Human movement:** While protocols have been developed to isolate and test any people displaying signs of illness at cross-border crossings (e.g., http://www.cdc.gov/vhf/ebola/hcp/index.html), protocols are also needed to manage illegal immigrant investigation and holding protocols. These individuals may not have travel documents indicating country visitation or citizenship. Management of these immigrants is often undertaken by multiple agencies (immigration, police, and defense forces). Appropriate training and procedures must be identified to address the multiagency nature of the activity and to allow for safe and respectful management of such individuals during times of heightened concern over human mobility and EBOV spread. Controls on borders must be done securely but in a manner that allows movement of critical supplies to affected regions.

4. **Need for continuing molecular epidemiological outbreak assessments:** Access to samples from the current outbreak is challenging, given the already-impossible burden placed on health staff active in the outbreak site. However, any samples and/or DNA sequence data available should be made accessible to the public health community as soon as possible in order to allow molecular investigations to advance. This will facilitate refinement of our understanding of transmission pathways (e.g., through determining transmission networks) and public health implications, among other areas of need. This information is urgently needed to address the challenge of containing this current outbreak and identifying appropriate control measures.

5. **Modelling tools and data gaps:** Modelling may provide essential information on potential scenarios for outbreak progression, intervention design, and logistics planning [107]. Data gaps in the outbreak region have been significant, however, limiting the full use of this tool set and our ability to address operational needs. Funding priorities for Ebola and other health research in Africa should be outcome-oriented and directed at addressing identified data gaps that are key to prevention and control in order to address immediate needs. Agent-based approaches can incorporate complex cultural and behavioral norms and can be used to direct data collection in data-poor environments [108]. Social media data are often used in public health, both in tracking infections and in delivery of health messages [109,110]. Lack of internet access in the current outbreak requires innovative approaches that will allow bridging of these essential data gaps and delivery opportunities for health messages. Two types of modelling efforts will be important, one that engages emergent

needs in an outbreak and a second directed at understanding broader elements of the epidemic and preventing future outbreaks. The scope and focus of each are complementary and allow scalable assessments of outbreak needs, both present and future.

6. **Bushmeat movement and use within Africa—increasing our ability to prevent spillover:** Wildlife smuggling and bushmeat trafficking occur extensively regionally and internationally. Wildlife meat is often deboned and skinned to decrease the likelihood of detection, and can be mistakenly identified as livestock meat. Protocols need to be developed for the safe seizure of suspected or known wildlife products at border crossings or elsewhere in country. Patterns of illegal bushmeat trafficking within and between African countries should be a priority area of investigation and areas of increased risk identified as best as possible for purposes of future outbreak prevention.

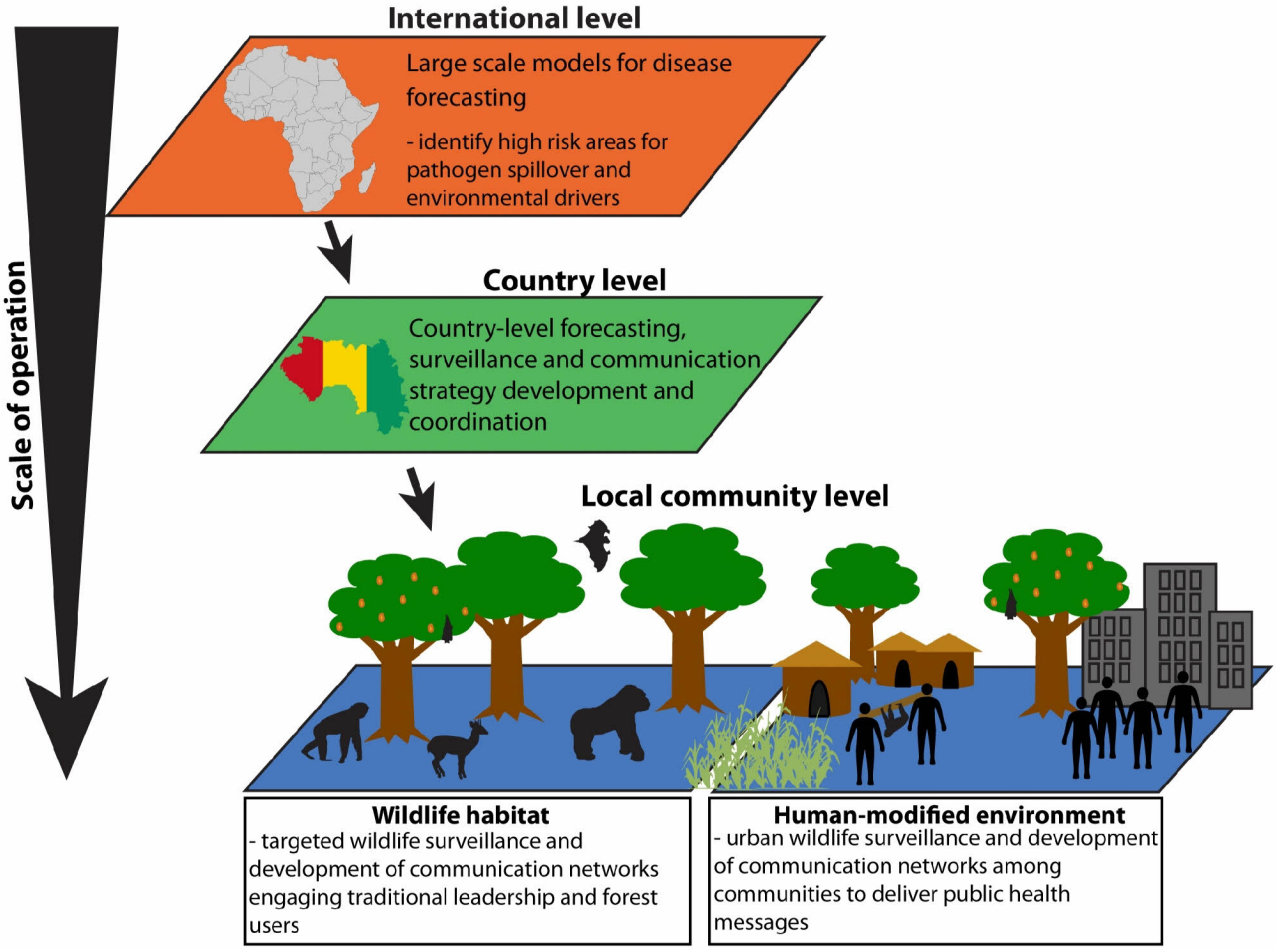

Fig 8. Schematic Ebola early-warning system. Development of any early-warning system for the prevention of future Ebola outbreaks will require a multiscaled effort that spans the international level down to the community, engaging partnerships between and within levels. The most important element of surveillance will be the effective engagement of local communities in regions of concern. A community-driven wildlife surveillance strategy should be designed through participatory approaches, driven by traditional leaders in partnership with country governments. Developed communication networks would need to engage forest users regarding observations of deceased or sick wildlife, in particular those species associated with Ebola outbreaks previously. Sociological assessments and community consultation would be needed to identify barriers to reporting dead or sick wildlife and development of appropriate educational approaches and other social interventions. While international assistance will be important, government and community ownership of the process at the national and local level will be important for sustainability. Research into Ebola reservoir and transmission dynamics will be essential to refining surveillance approaches.

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7. **Multiscale early-warning systems and future preparedness strategies:** While international and regional modelling efforts provide important tools for forecasting risk zones, community-based surveillance will be necessary to effectively identify Ebola emergence in wildlife (detection of death and/or sickness) before outbreaks occur at the local level (Fig 8). Public health education will be important in reducing behaviors that increase risk of spillover from wildlife sources.
8. **Global Public Health Education Needs:** Public health education regarding Ebola dynamics and transmission is not only needed urgently in Africa but, increasingly, around the world. In the US, public panic appears to be escalating, and there is the risk that choices may be driven by fear rather than fact [31]. A focused program of communication from public health officials is urgently needed and should involve multiple outlets such as radio, television, and social media platforms. These communications should provide factual information concerning the management of Ebola risk, tailored to the target population.

Africa is a changing landscape, and our approaches will have to engage the complexities of the region and community livelihoods. It is clear that many factors could have contributed to the emergence of Ebola in West Africa. Increasing population size, social unrest, and poverty have undoubtedly influenced both the explosive and sustained nature of this epidemic and our collective inability to contain it. We will need to rethink our approach to disease emergence events in low-resource areas, where significant knowledge gaps exist and operational barriers impede isolation and control efforts. The doctors, nurses, public health officials, non-governmental organizations (NGOs), and political leaders are presently challenged with on-the-fly responses to public health emergencies in a low-resource area and are to be congratulated for their ingenuity and perseverance. The real partnerships that are emerging among community leaders, NGOs, governments, and international agencies must be encouraged and facilitated to the greatest possible extent.

Key Learning Points

- Significant political, social, and environmental changes have occurred in West Africa, likely contributing to the emergence of the most deadly Ebola outbreak in history.
- Similarity in outbreak characteristics (including R0, symptoms, incubation time, and serial time) between West Africa and previous Ebola outbreaks suggests that there has not been any significant change in the virus affecting transmissibility.
- Information collection and communication remain a challenge in resource-poor settings and specific strategies and tools will need to be developed to allow rapid identification and response within the context and constraints identified in the local environment.
- Integrated approaches involving both human and animal health must be developed that engage the research, law enforcement, and policy environments within these local settings.

Top Five Papers

1. Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature. 2005;438:575–576.
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Seasonal Pulses of Marburg Virus Circulation in Juvenile *Rousettus aegyptiacus* Bats Coincide with Periods of Increased Risk of Human Infection

Brian R. Amman[1], Serena A. Carroll[1], Zachary D. Reed[1], Tara K. Sealy[1], Stephen Balinandi[1], Robert Swanepoel[2¤a], Alan Kemp[2], Bobbie Rae Erickson[1], James A. Comer[1], Shelley Campbell[1], Deborah L. Cannon[1], Marina L. Khristova[3], Patrick Atimnedi[4], Christopher D. Paddock[5], Rebekah J. Kent Crockett[6], Timothy D. Flietstra[1], Kelly L. Warfield[7], Robert Unfer[7], Edward Katongole-Mbidde[8], Robert Downing[9], Jordan W. Tappero[9], Sherif R. Zaki[5], Pierre E. Rollin[1], Thomas G. Ksiazek[1¤b], Stuart T. Nichol[1], Jonathan S. Towner[1]*

1 Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **2** National Institute of Communicable Diseases, Special Pathogens Unit, Johannesburg, South Africa, **3** Biotechnology Core Facility Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **4** Uganda Wildlife Authority, Kampala, Republic of Uganda, **5** Infectious Disease Pathology Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **6** Division of Vector-borne Diseases, Arbovirus Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **7** Integrated BioTherapeutics, Gaithersburg, Maryland, United States of America, **8** Uganda Virus Research Institute, Entebbe, Republic of Uganda, **9** Global AIDS Program, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

Abstract

Marburg virus (family *Filoviridae*) causes sporadic outbreaks of severe hemorrhagic disease in sub-Saharan Africa. Bats have been implicated as likely natural reservoir hosts based most recently on an investigation of cases among miners infected in 2007 at the Kitaka mine, Uganda, which contained a large population of Marburg virus-infected *Rousettus aegyptiacus* fruit bats. Described here is an ecologic investigation of Python Cave, Uganda, where an American and a Dutch tourist acquired Marburg virus infection in December 2007 and July 2008. More than 40,000 *R. aegyptiacus* were found in the cave and were the sole bat species present. Between August 2008 and November 2009, 1,622 bats were captured and tested for Marburg virus. Q-RT-PCR analysis of bat liver/spleen tissues indicated ~2.5% of the bats were actively infected, seven of which yielded Marburg virus isolates. Moreover, Q-RT-PCR-positive lung, kidney, colon and reproductive tissues were found, consistent with potential for oral, urine, fecal or sexual transmission. The combined data for *R. aegyptiacus* tested from Python Cave and Kitaka mine indicate low level horizontal transmission throughout the year. However, Q-RT-PCR data show distinct pulses of virus infection in older juvenile bats (~six months of age) that temporarily coincide with the peak twice-yearly birthing seasons. Retrospective analysis of historical human infections suspected to have been the result of discrete spillover events directly from nature found 83% (54/65) events occurred during these seasonal pulses in virus circulation, perhaps demonstrating periods of increased risk of human infection. The discovery of two tags at Python Cave from bats marked at Kitaka mine, together with the close genetic linkages evident between viruses detected in geographically distant locations, are consistent with *R. aegyptiacus* bats existing as a large meta-population with associated virus circulation over broad geographic ranges. These findings provide a basis for developing Marburg hemorrhagic fever risk reduction strategies.

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* E-mail: jit8@cdc.gov

¤a Current address: Zoonoses Research Unit, Department of Medical Virology, University of Pretoria, Pretoria, South Africa
¤b Current address: Galveston National Lab, Department of Pathology, University of Texas Medical Branch, Galveston, Texas, United States of America

Introduction

Marburg virus (family *Filoviridae*), is the etiologic agent of Marburg hemorrhagic fever (MHF), a severe disease associated with person-to-person transmission and high case fatality. The virus was discovered in August 1967 when simultaneous outbreaks of MHF occurred in laboratory workers in Germany and Yugoslavia [1,2]. The source of the virus was associated with importation of infected African green monkeys (Cercopithecidae: formerly *Cercopithecus aethiops*; currently *Chlorocebus tantalus* [3]) consigned from Uganda to Europe for use in the laboratories where the outbreaks occurred [4].

Since its discovery, the sporadic nature of Marburg virus outbreaks and the diverse history of human exposures have made it difficult to definitively trace the virus to its natural source, but mounting evidence has shown a recurrent link to caves or mines,

Author Summary

Marburg virus, like its close relative Ebola virus, can cause large outbreaks of hemorrhagic fever with case fatalities nearing 90%. For decades the identity of the natural reservoir was unknown. However, in 2007 Marburg viruses were isolated directly from Egyptian fruit bats (*Rousettus aegyptiacus*) that inhabited a Ugandan gold mine where miners were previously infected. Soon after, two tourists became infected with Marburg virus after visiting nearby Python Cave, a popular attraction in Queen Elizabeth National Park, Uganda. This cave also contained *R. aegyptiacus* bats (~40,000 animals). These events prompted a long-term investigation of Python Cave to determine if, 1) *R. aegyptiacus* in the cave carried infectious Marburg virus genetically similar to that found in the tourists, and 2) what ecological factors might influence virus spillover to humans. In the study, we found that, 1) approximately 2.5% of the bat colony is actively infected at any one time and that virus isolates from bats are genetically similar to those from infected tourists, and 2) specific age groups of bats (juveniles~six months of age) are particularly likely to be infected at specific times of the year that roughly coincide with historical dates of Marburg virus spillover into humans.

leading investigators to suspect bats as a likely reservoir. In early February 1975, the second known outbreak of MHF occurred after two tourists traveled through Zimbabwe and reported sleeping in rooms with bats and visiting Chinhoyi caves in the days before developing symptoms [5]. In January 1980, and then again in August 1987, two patients contracted MHF after visiting a cave complex with large bat populations on Mt Elgon, Kenya. From 1998–2000, a protracted outbreak occurred at the Goroumbwa mine in Durba village in northeast Democratic Republic of Congo (DRC) and consisted of multiple short chains of virus transmission among gold miners and their families [6]. A concomitant ecological investigation found the mine to be populated with large numbers of bats of several species, three of which were later found to have evidence of Marburg virus infection, most notably the Egyptian fruit bat *Rousettus aegyptiacus* (order Chiroptera: family Pteropodidae) which had the highest prevalence (20.5%) of antibody to the virus [7]. In 2005, a healthcare center-based outbreak in Uige, northern Angola, became the first MHF outbreak to be detected on the west coast of Africa and the largest MHF outbreak on record [8]. The origin of the Angola outbreak was never determined, but that same year in nearby Gabon, a survey of 1,100 bats representing 10 bat species found only the cave-dwelling *R. aegyptiacus* to be positive for evidence of Marburg virus infection [9]. However, in both the Gabon and Durba DRC studies, scientists were unable to isolate Marburg virus from infected bat tissues.

In July and September 2007, MHF re-emerged in gold miners, this time in southwest Uganda at the Kitaka mine which is approximately 1,280 km from Durba. Here, genetic evidence showed two independent virus introductions from the natural reservoir into humans. A mark-recapture study estimated the mine to populated by over 100,000 *R. aegyptiacus*, from which five genetically diverse Marburg virus isolates were obtained from bats collected over an eight month period, demonstrating that *R. aegyptiacus* can naturally harbor infectious Marburg virus and that multiple lineages of virus can persist in a same bat colony for an extended period [10].

A year later, in late June 2008, MHF again occurred in southwest Uganda. This case involved a Dutch tourist who became fatally infected following a visit to Python Cave in Queen Elizabeth National Park (QENP) [11]. Python Cave is a popular tourist attraction 50 linear kilometers from the Kitaka mine and is known for the large African rock pythons that give the cave its name, but more importantly, its large *R. aegyptiacus* colony upon which the snakes feed. The publicity from the Dutch MHF case resulted in the retrospective identification of a second, non-lethal, MHF case associated with Python Cave. This individual was an American tourist who visited the bat colony in late December 2007 and developed MHF symptoms soon after returning home to Colorado, USA [12].

Together, these epidemiologic and laboratory data indicate *R. aegyptiacus* is a natural reservoir for Marburg virus. However, important questions remain such as how the virus naturally persists in these bats, and what ecological drivers cause occasional spillover from bats to humans. In the present study, we report a multi-year investigation of natural Marburg virus circulation among *R. aegyptiacus* in southwest Uganda, with emphasis on bats inhabiting Python Cave. Our data show a dynamic pattern of Marburg virus transmission that produces cyclical fluctuations in active infections associated with defined age cohorts of the bat population.

Results/Discussion

Description of Python Cave and bat collections

In response to the infection of the American and Dutch tourists, a series of four ecological investigations were conducted at Python Cave from August 2008 through November 2009. The goals of this study were to 1) determine if Marburg virus infected bats were present in the cave, and if so, what species of bat; and 2) determine what ecological factors, if any, may have led to the human infections. *Rousettus aegyptiacus* breed twice a year, becoming pregnant around November and May and giving birth in February and August, respectively (gestation period is approximately 105–107 days based on captive observations) [13]. The bat collections were scheduled during peak breeding or birthing periods (August 2008, February 2009, August 2009, November 2009) and were designed to complement two previous studies at the nearby Kitaka mine which were also carried out during similar peak times of either the birthing or breeding seasons (August 2007 and May 2008 respectively). Based on comparisons to the Kitaka mine, which contained over 100,000 *R. aegyptiacus* and a large number of smaller insectivorous bats (*Hipposiderous spp.*), the bat population at Python Cave was estimated to be at least 40,000 animals, and *R. aegyptiacus* was the sole chiropteran inhabitant of the cave.

Python Cave is actually a tunnel open at both ends, and is approximately 15 meters (m) long and 12 m wide, formed by a subterranean stream that undercut a land bridge spanning a small gorge. The height of the interior is variable, ranging from 3.5 m to nearly 5 m due to the boulder strewn floor, and the cave contains numerous nooks, crevices and hidden chambers, with nearly every square centimeter of 'hanging space' used by the bats. The limited space forces bats to occupy sunlit ledges of the gorge on either side of the tunnel openings. Most juvenile bats were observed roosting in these more peripherally located pockets and ledges near the ground, both inside and outside of the tunnel proper while adults tended to occupy the darker interior. These juvenile bats were also observed roosting on the sides of the larger boulders and in holes on the cave floor.

In addition to the bats, other vertebrate fauna observed in the cave included at least two large African rock pythons (*Python sebae*), and several forest cobras (*Naja melanoleuca*). Also observed visiting

Table 1. Summary of *Rousettus aegyptiacus* caught at Python Cave displayed by class, and PCR, virus isolation, and ELISA results.

		Captures	PCR +	Isolates	Ab +
Female	Adult	499	4	2	139
	Non-adult	299	17	2	20
	Total	**798**	**21**	**4**	**159**
Male	Adult	494	7	—	75
	Non-adult	330	12	3	16
	Total	**824**	**19**	**3**	**91**
Total		**1622**	**40**	**7**	**250**

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the cave were African fish eagles (*Haliaeetus vocifer*), palm-nut vultures (*Gypohierax angolensis*), Nile monitor lizards (*Varanus niloticus*) and olive baboons (*Papio anubis*). Further, a variety of invertebrates were found, most notably argasid ticks (Family Argasidae) on the cave walls, nycteribiid flies (Family Nycteribiidae) in the bat pelage, and fresh water crabs (Crustacea: Decapoda) in the subterranean stream beneath the cave floor.

Over the four sampling periods at Python Cave, 1,622 *R. aegyptiacus* were captured and tested for Marburg virus. Both genders were represented nearly equally (Table 1). Of the 798 females captured, 449 were of active breeding age evidenced by having an attached pup, being pregnant or having enlarged nipples indicative of previous lactation. Of the 824 males captured, 453 were scrotal. The majority (61%) of the total captures (n = 1,622) were adults (n = 993; forearm length >89 mm) while the remainder consisted of volant juveniles (n = 417) or newborn pups (n = 212).

Evidence of Marburg virus infection by Q-RT-PCR and virus isolation from bat tissues

Viral RNA extracted from pooled liver and spleen samples were tested for Marburg virus RNA using a real-time Q-RT-PCR assay designed to detect all known strains of Marburg virus [10]. Of the 1,622 bats captured, 40 (2.5%) were actively infected as evidenced by having detectable Marburg virus RNA (Q-RT-PCR positive). A population estimate of 40,000 bats combined with an infection level of 2.5% estimates approximately 1,000 actively infected bats to reside inside this popular tourist destination at certain times of the year. Several other tissues tested positive for Marburg virus RNA (Table 2) and always in conjunction with positive liver and spleen samples, including kidney ($n = 2$), colon and rectum ($n = 5$), lung ($n = 8$), heart ($n = 3$), intestine ($n = 3$) and blood ($n = 2$). The array of virus-infected tissues indicates that *R. aegyptiacus* inhabiting Python Cave are probably in diverse stages of infection. Some bats, (e.g. bat #843 in Table 2) appear acutely and systemically infected as evidenced by simultaneous infection of lung, liver/spleen, kidney, colon, mid-gut, heart and blood. The Marburg virus-specific RNA loads found in blood of bats #843 and #1175 were very low (Ct values between 30–39; indicating lower amounts of viral RNA) and could not explain the higher RNA levels seen in the other infected tissues (Ct values between 20–30; indicating higher amounts of viral RNA). All bats with multiple Marburg virus-positive tissues were also positive by testing of pooled liver/spleen suggesting that liver and spleen remain the best target tissues for identifying Marburg virus-infected *R. aegyptiacus*. Finding Marburg virus in tissues from lung, kidney, colon, and mid-gut raises the possibility of virus shedding through an oral, fecal, or urinary route(s). One bat had Marburg virus-positive reproductive tissue (uterus/ovary) which, given the previous discovery of Ebola virus in reproductive tissue of infected humans [14–16] and active Marburg virus transmission via semen [17], raises the possibility of sexual transmission among bats. The potential involvement of arthropod vectors has not been ruled out, although limited

Table 2. Summary of *Rousettus aegyptiacus* found positive for Marburg virus in multiple tissues by Q-RT-PCR.

Date	Bat #	Sex	Age	Li/Sp	Heart	Lung	Kidney	Colon	Repro	Intestine*	Blood
Aug 09	843	Male	J	++++	+	++++	++	++	–	+++	++
Aug 09	849	Female	J	+	–	–	–	+	–	++	–
Aug 09	907	Female	J	+	–	–	–	–	–	+	–
Aug 09	914	Female	J	++	–	+	+	–	–	–	–
Aug 09	934	Female	J	+	–	–	–	++	–	–	–
Aug 09	960	Male	J	+	–	++	–	+++	–	–	–
Aug 09	1134	Female	J	+	–	+	–	–	–	–	–
Aug 09	1175	Male	J	+++	–	–	–	–	–	–	+
Nov 09	1232	Female	J	++	+	+	–	–	–	–	–
Nov 09	1261	Male	A	++	–	+	–	–	–	–	–
Nov 09	1304	Female	J	+++	++	++	–	+	+	–	–
Nov 09	1368	Male	J	++	–	+	–	–	–	–	–

For reference, approximate TCID50 values for positive tissues were derived from a standard curve of diluted stock virus (371Bat Uga 2007) assayed using the identical Q-RT-PCR assay as that used for the tissues.
J = juvenile bat (non-pup; forearm length ≤89 mm).
A = adult bat (forearm length >89 mm).
++++ = Ct 20–25 = (50,000–1,500,000 $TCID_{50}$/ml).
+++ = Ct 25–30 = (2000–50,000 $TCID_{50}$/ml).
++ = Ct 30–35 = (100–2000 $TCID_{50}$/ml).
+ = Ct 35–39 = (5–100 $TCID_{50}$/ml).
*Pool of 3 tissue sections.
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numbers of argasid ticks (14 pools of 10–20 ticks) collected thus far from the cave were negative for Marburg virus RNA by Q-RT-PCR.

From the Q-RT- PCR positive bats at Python Cave, seven genetically distinct Marburg virus isolates (Table 1) were obtained directly from homogenized liver/spleen tissue, and for one bat (#843) virus was additionally isolated from lung and blood (viremia). These virus isolates, combined with those from five bats captured at the Kitaka mine, bring to 12 the total number of bats from which Marburg virus has been isolated. In fact, Marburg virus was isolated at least once from each *R. aegyptiacus* collection expedition in Uganda, including those at the Kitaka mine [10], with the exception of the 2009 February/March Python Cave collection, which yielded no virus isolate. There were no significant differences in the ability to isolate virus from either Q-RT-PCR positive adults (2/11, 18.18%) or juveniles (5/28, 17.85%; $t=-0.023$, $p>.98$), or likewise, from males (3/19, 15.79%) or females (4/20, 20.0%; $t=.334$, $p>.70$). Successful isolation of Marburg virus roughly correlated with samples that had Ct values of 30 or less (>2000 $TCID_{50}$/ml).

Immunohistochemical analyses

Immunohistochemical analysis (IHC) was performed on formalin fixed liver and spleen tissues from all Q-RT-PCR positive bats and an approximate equal number of negative bats. Of the 40 Marburg virus positive bats, four (10%) were positive via IHC in liver, one of which (Bat #843) was additionally positive in spleen. All Q-RT-PCR positive heart, lung, kidney, colon and mid-gut tissues shown in Table 2 with Ct values less than 35 (virus loads $>\sim100$ $TCID_{50}$/ml), were additionally tested by IHC, but none were positive for Marburg virus antigen. There was no evidence of any pathology apparent during necropsies or IHC analysis that could be attributed directly to infection with Marburg virus. Moreover, there were no signs of overt morbidity or mortality witnessed during the capture or processing of the bats, including those actively infected with Marburg virus. However, the cave environment is such that dead or dying bats might not be visible for long periods of time due to predation, guano accumulation, and the large detritivore community living in the cave.

Phylogenetic relationship of Marburg virus sequences from bats and humans and evidence of long distance *R. aegyptiacus* movement

Full-length genome sequences (19,114 bp) were determined from all seven of the Python Cave Marburg virus bat isolates. Two isolates (164QBat Uga 2008 and 1328QBat Uga 2009) closely match the sequence of the virus isolate obtained from the Dutch MHF case (01Uga/Net 2008; Fig. 1) based on a Bayesian analysis. Unfortunately, no virus was isolated from the American tourist, but the sequence from small portions of the NP and VP35 genes were obtained from clinical material following amplification by nested RT-PCR. The sequences were concatenated into a single ~700 nt sequence and analyzed with corresponding Marburg virus sequences from bats and humans using similar Bayesian methods. As expected, multiple Marburg virus sequences from Python Cave bats closely match that of the American tourist (Fig. 2). Further, these two analyses produced phylogenies showing that the entire known genetic spectrum of Marburg virus, >20% nucleotide diversity, can be found circulating in Python Cave at any one time. This finding is consistent with *R. aegyptiacus* representing a *bona fide* long term reservoir species for the virus.

The fact that several of the Marburg virus sequences from Python Cave and Kitaka mine are similar to sequences obtained from distant regions of sub-Saharan Africa including Gabon (48Gab 2005, 31Gab 2005, and 96Gab 2006) and Zimbabwe (OzoZim 1975) suggest that there is considerable animal movement over long distances and exchange of infectious virus through a network of *R. aegyptiacus* colonies that span the continent. As proof of direct animal movement between *R. aegyptiacus* bat colonies, a numbered collar was found at Python Cave in August 2008 that had been initially placed on an adult female *R. aegyptiacus* bat at the Kitaka mine during the mark and recapture study three months earlier [10]. The Kitaka mine and Python Cave are separated by roughly 50 linear kilometers and separated by tracts of dense forest and zones of agricultural activity. In South Africa, marked *R. aegyptiacus* have been shown to move up to 32 km between roosting sites and in one instance, a marked female relocated to a site 500 km away [18]. Additional evidence of direct movement between colonies was found when a second *R. aegyptiacus* bat, marked as a male juvenile at the Kitaka mine in 2008, was captured at the Python Cave as an adult in August of 2009, a full 15 months after the initial capture and marking.

Older juvenile bats are most likely to be actively infected with Marburg virus

In the initial 2007 Kitaka mine investigation [10], a significantly higher proportion of juvenile bats were found to be actively infected than were adults (12% vs 4.2% respectively), yet in the follow-up study at the same location nine months later (in May 2008), the proportions of infected juveniles and adults were slightly inverted (1.7% vs 5.7% respectively) [10]. From these early data, it was hypothesized that perhaps the reason for the difference in infection prevalence resided in factors related to the age of the juvenile cohorts, being six months old during the birthing seasons (August and February) yet only three months old during the breeding seasons (May and November). At the time of capture, older juveniles (six months old) would have been weaned for at least four months, fully independent and without any residual Marburg-specific maternal antibody if they were born to an antibody positive mother. In contrast juveniles caught during breeding seasons (May and November) would be roughly three months old, barely independent, and newly released from the physically occlusive protection of their mother. Newborn pups remain attached to the nipple and well under the wing of the mother for the first six weeks of their lives and then remain in close contact, occasionally clinging to the mother's back for an additional two weeks (Towner and Amman personal observations of captive *R. aegyptiacus* bats).

Analysis of the Python Cave Q-RT-PCR data reveals a seasonal age bias among Marburg virus-infected bats which correlates with that observed at Kitaka mine [10]. Of the 40 total Q-RT-PCR positive bats from Python Cave, 29 (of 627 total) were juveniles compared to 11 (of 994 total) adults ($t=3.898$, $p<.001$). When the active infection data from the Kitaka mine and Python Cave investigations are combined and sorted into three age categories, young juveniles, old juveniles and adults, a reproducible age-linked infection pattern emerges (Fig. 3a). Levels of active infection among young juveniles remain around 2–3% (8/301, 2.65%) and increase to 10–15% by six months of age (30/241, 12.4%; $t=-4.212$, $p<.001$). Adults by contrast maintain a relatively constant level of active infection (Fig. 3b) ranging from 2–5% (33/1467, 2.4%), irrespective of season (breeding season = 11/305, 3.6%; and birthing season = 22/1163, 1.9%; $t=1.508$, $p>.13$%). Interestingly, no evidence of vertical transmission was found. In one instance, a Q-RT-PCR positive mother was identified with an Q-RT-PCR negative pup. Moreover, all pups from either Kitaka

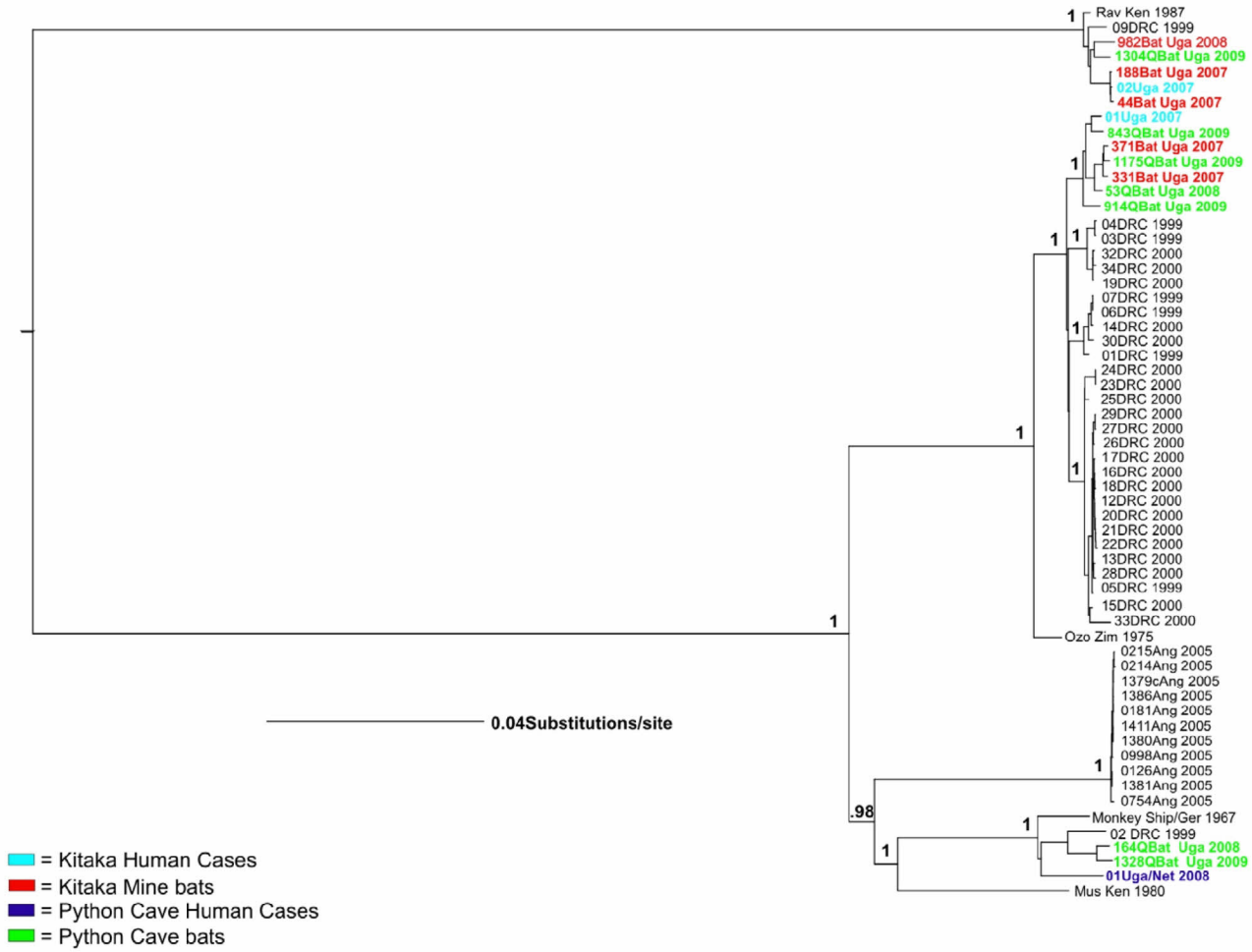

Figure 1. Bayesian phylogeny of full length Marburg genome. Phylogenetic results from a Bayesian analysis on full-length Marburg virus genome sequences from 12 Marburg bat isolates, 3 recent Ugandan human isolates from the two Kitaka miners (01Uga 2007, 02Uga 2007), and the Dutch tourist (01Uga/Net 2008), as well as 45 historical isolates (Table S2 for GenBank accession numbers). Posterior probabilities above .50 are shown above the appropriate nodes. Marburg virus sequences from human cases from Kitaka mine (Uganda 2007) in are in orange, sequences from human cases from Python Cave (2008 Uganda) are in blue, sequences from Kitaka Mine bats are in red, and sequences from Python Cave bats are in green.
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mine or Python Cave (n = 223) tested uniformly negative for active Marburg virus infection.

Together, these data present a dynamic picture of natural Marburg virus circulation in which juveniles are exposed to the virus at an early stage of their development following independence at three months of age and increasing up through their first six months of life. Once in the adult population after seven to eight months of age, the incidence of infection apparently drops off for reasons not currently understood and levels out to a more constant rate that is independent of season. We are currently developing reliable measures for sub-adult age classification, but until they are complete, tracking the younger age cohorts beyond six to seven months of age remains difficult.

The overall pattern of horizontal transmission is supported by serological data from the Python Cave bats in which Marburg virus-specific IgG antibody prevalence increases with age starting from 4.1% (10/242) among young juveniles and increases to 14.8% (26/175) among older juveniles and finally reaches 21.5% (214/993) in adults. The lower infection levels observed in young juveniles is likely due to lack of physical opportunity for exposure to other members of the population perhaps aided by maternal antibody protection for those pups born to antibody positive mothers. In our analyses, all pups of antibody positive mothers ($n = 20$) were themselves antibody positive. It is unknown if maternal antibody is actually protective.

We speculate that the introduction of Marburg virus into the juvenile bat population may also be influenced by the positioning of bat groups within the cave. On every occasion, segregation of juveniles (non-pups) from adults was witnessed with juvenile bats generally pushed to the periphery of the cave away from the center where it is darkest. At the periphery, juveniles were observed roosting tightly together primarily in small holes or on the sides of large boulders on the cave floor. Occasionally small groups of juveniles could be found low on the walls but outside the cave in filtered sunlight. The cave floor contains copious amounts of accumulated guano (feces and urine) that are continually refreshed by new deposits. Should virus be shed through bat excretions, the physical positioning of juvenile bats directly underneath the adult bats would make juvenile bats particularly susceptible to virus exposure. Unfortunately, testing of limited (<100 samples) urine

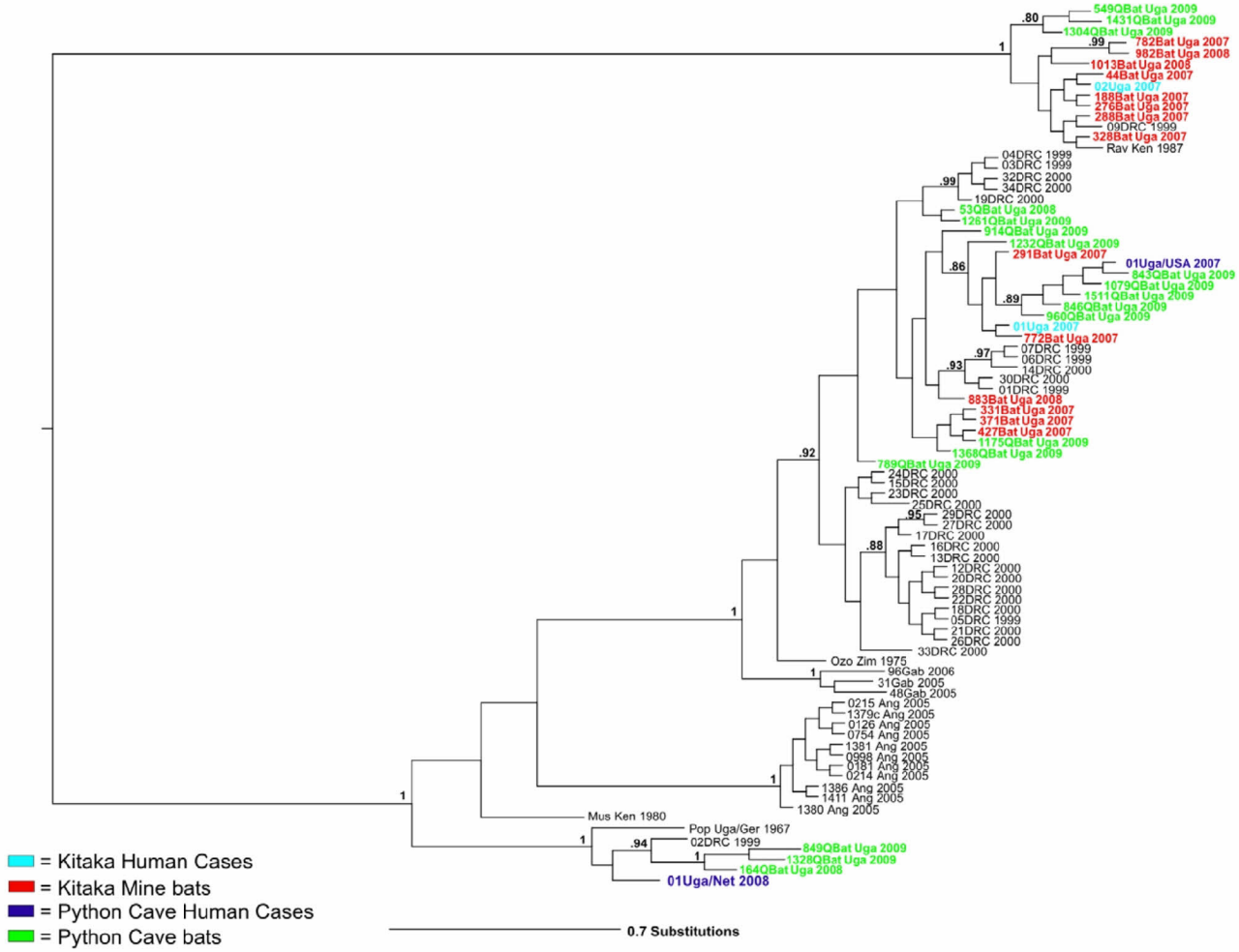

Figure 2. Bayesian phylogeny of Marburg NP and VP35 genes. Phylogenetic results from a Bayesian analysis on concatenated NP and VP35 sequence fragments obtained from bat specimens, historical isolates (45), and the recent Ugandan human samples (01Uga 2007, 02Uga 2007, 01Uga/Net 2008) as well as the American tourist (01Uga/USA 2007), for which there was no isolate, only partial Marburg virus sequence (Table S2 for GenBank accession numbers). Sequences 846QBat_Uga_2009, 849QBat_Uga_2009, 1079QBat_Uga_2009, 1261QBat_Uga_2009, 1328QBat_Uga_2009, and 1511QBat_Uga_2009 represent NP only. Posterior probabilities above .50 are shown above the appropriate nodes. Marburg virus sequences from human cases from Kitaka mine (Uganda 2007) in are in orange, sequences from human cases from Python Cave (2008 Uganda) are in blue, sequences from Kitaka Mine bats are in red, and sequences from Python Cave bats are in green.
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and fecal samples for viral RNA has not yet yielded positive results, probably due to persistent Q-RT-PCR inhibitors that have thus far hindered our ability to detect Marburg virus RNA in experimentally spiked guano samples in the laboratory (data not shown). Nevertheless, finding of Marburg virus-positive kidney, colon/rectum, and intestine samples, suggests virus shedding through excreta may well occur.

As the juveniles age and are recruited into the adult population or disperse to other caves or suitable sites, the low lying roosting areas are repopulated by the next pulse of newly weaned juveniles. These juveniles in-turn become infected, spreading the virus primarily amongst themselves until they too disperse or move into the adult population. This cycle continues season after season to perpetuate virus transmission within the colony. The pattern of continual circulation of the virus within the population coupled with the continued lack of any overt morbidity and mortality in infected bats is consistent with expectations for *Rousettus aegyptiacus* being a natural reservoir for Marburg virus.

Seasonal clustering of spillover events to humans coincide with peaks of infection in juvenile bats

The approximate dates of 13 suspected Marburg virus spillover events were determined from the literature (Table 3), seven of which were linked directly to subterranean gold mining activities at the bat-inhabited mines in Durba, DRC from 1994–1997 [6] and Ibanda, Uganda 2007 [10]. Five spillover events involved tourists with defined dates of visitation to caves containing *R. aegyptiacus*, in the weeks just before the onset of MHF symptoms. The original 1967 outbreak was also included, and for that, a date was chosen that was one incubation period (three weeks) prior to the first shipment of infected monkeys that arrived in Frankfurt, Germany on 21 July 1967 (via London Heathrow airport) and further distributed within Germany (Marburg and Frankfurt) and to Belgrade, Yugoslavia [19]. When all 13 Marburg virus spillover events are listed by month of occurrence, the data show a temporal clustering of human infections, coinciding with the summer (mid-June through mid-September) and winter months (mid-December

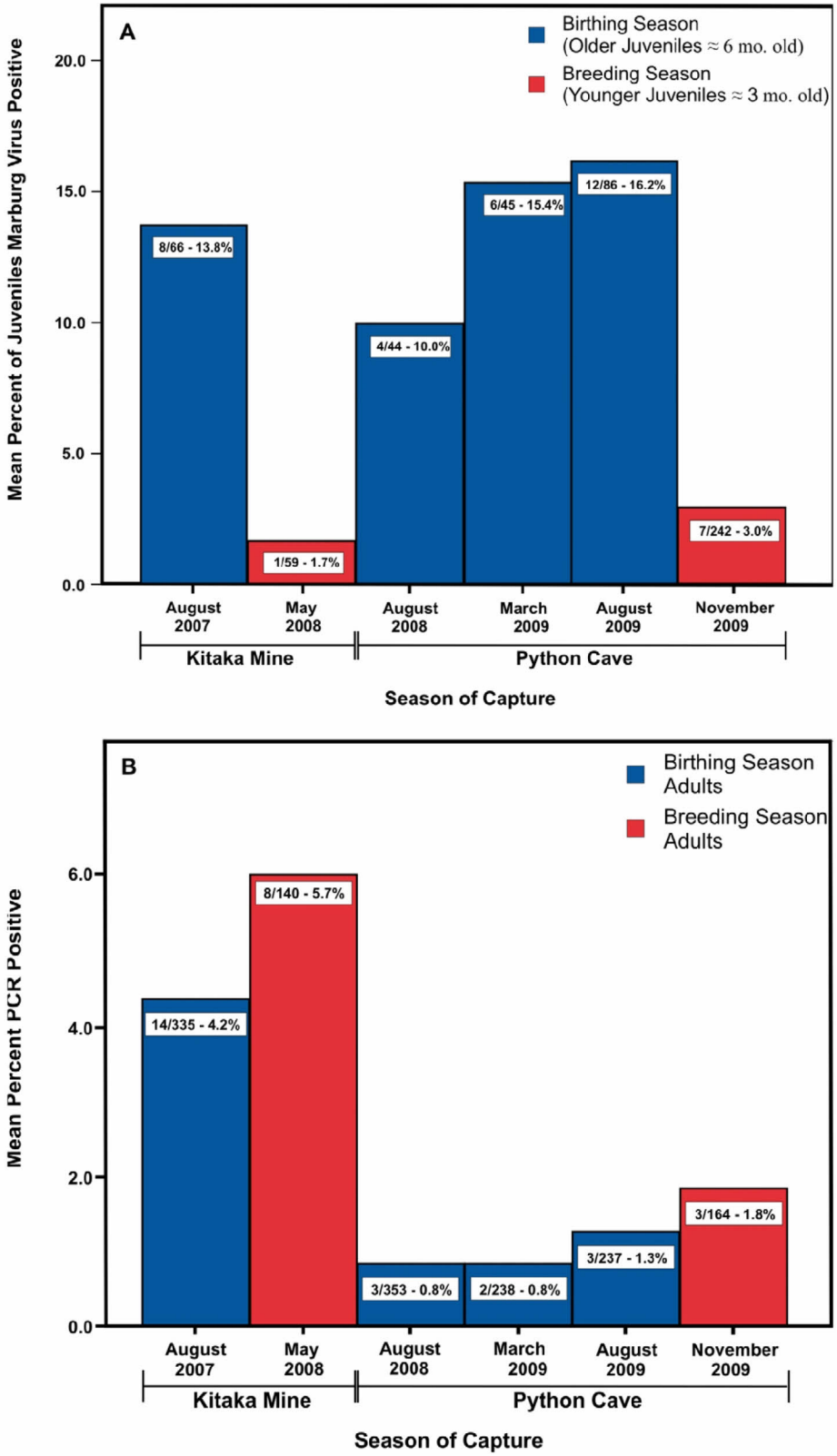

Figure 3. Percent active infection among older and younger juvenile bats and adults. (A) Histogram showing the percent of juvenile bats from Kitaka Mine and Python Cave actively infected (Q-RT-PCR+) with Marburg virus during breeding and birthing seasons. (B) Histogram of the percent of adult bats from Kitaka Mine and Python Cave actively infected (Q-RT-PCR+) with Marburg virus during breeding and birthing seasons.
doi:10.1371/journal.ppat.1002877.g003

Table 3. Historical Marburg spillover events with dates of initial exposure excluding the 2005 Angola outbreak because the initial exposure date was never identified.

Date of Exposure	Country	Citation
30 Jun 1967	Germany Yugoslavia via Uganda	Extrapolated by subtracting one incubation period (21 days) from the date of the shipment received listed in [4,19].
1–9 Feb 1975	South Africa via Zimbabwe	Index case traveled in Rhodesia Feb 1–9, admitted on 15 Feb 1975 [31].
25 Dec 1980	Kenya	Kitum (Elgon) Cave 25 December –15 days before illness [32].
1 Aug 1987	Kenya	Kitum Cave – 9 days before illness [33].
Feb 1994	DRC - Durba	Identified in Fig. 3 of Bauch et al [6].
Jul 1994	DRC - Durba	Identified in Fig. 3 of Bauch et al [6].
Sep 1995	DRC - Durba	Identified in Fig. 3 of Bauch et al [6].
Mar 1996	DRC - Durba	Identified in Fig. 3 of Bauch et al [6].
May 1997	DRC - Durba	Identified in Fig. 3 of Bauch et al [6].
10 June 2007	Uganda	Epidemiological data obtained during an outbreak investigation [34].
14 Sep 2007	Uganda	Epidemiological data obtained during an outbreak investigation [34].
25 Dec 2007	USA via Uganda	[12].
19 Jun 2008	Netherlands via Uganda	[11].

doi:10.1371/journal.ppat.1002877.t003

through mid-March) of the northern hemisphere. The majority of spillover events (7/13) involved resident African miners, suggesting that the clustering effect was not due to seasonal tourism. More importantly, when the dates of these 13 spillover events are compared to a sinusoidal curve derived from the field collection data showing the seasonal incidence of juvenile *R. aegyptiacus* infections (Fig. 4), a pattern of coincidence emerges. The sinusoidal curve has peaks and troughs that correspond to the beginning of the birthing and breeding seasons respectively, each separated by roughly three months, and whose peak heights reflect the average percentage of infected juveniles for each seasonal category. These data show that 11 of 13 (84.6%, Fisher's Exact Test $p<.05$) spillover events occurred during the three month periods encompassing each of the two biannual birthing seasons when juvenile bats are roughly 4.5–7.5 months old and most likely to be infected with Marburg virus. Moreover, when suspected (extrapolated) exposure dates for 52 primary cases (all miners and epidemiologically unlinked to any other human cases; Table S1) from the final MHF patient list from the 1998–2000 outbreak in Durba, DRC [6] are included in the analysis (Pierre Rollin and Robert Swanepoel; personal communication; Table S2), 54 of 65 (83.1% Fisher's Exact Test $p<.05$) spillover events occur during the same periods encompassing each of the biannual birthing seasons, further supporting the idea that these three-month periods may represent times of increased risk for exposure to Marburg virus. The contribution of young naïve bats to the overall population during these seasons is considerable. Based on a population estimate of 40,000 bats in Python Cave and 80% pregnancy of sexually active females [10,20], the number of births at Python Cave could easily exceed 20,000 pups a year (10,000 pups every 6 months). Many of those pups will become juveniles that are ultimately pushed to the periphery of the cave where they may be more likely to encounter humans.

We conclude that Marburg virus transmission within the *R. aegyptiacus* colony occurs year round at a baseline level, and that the months surrounding the peak birthing seasons represent times of increased infection among juveniles. Further, the coincidence of peak periods of juvenile bat infections with the historical clustering of individual spillover events to humans at similar times of the year suggests these seasonal periods might represent periods of heightened public health risk perhaps due to the positioning of the juvenile roosting sites within the cave. These data provide the first long-term monitoring of any filovirus circulating in nature and provide a foundation for understanding ecological drivers that may instigate MHF outbreaks.

Materials and Methods

Bat capture and processing

All procedures listed herein (including those referred to in Towner et al. [10]), were performed in accordance with an institutionally approved animal care and use protocol (animal use protocol 1731AMMULX approved by the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee). All aspects of the bat collections were undertaken with the approval of the Uganda Wildlife Authority and following the American Veterinary Medical Association guidelines on euthanasia and the National Research Council recommendations for the care and use of laboratory animals [21,22].

Without exception, protective equipment (PPE) standard for working with filoviruses in the field setting was used [23]. Briefly, all personnel donned double latex gloves, disposable Tyvek suit, rubber boots, fitted p100 respirators (3M) and eye protection (in the form of a full face shield or full-face respirator) prior to entering the cave. When appropriate, personnel used bite-resistant gloves, full face shields, caving helmets for head protection, and due of the presence of multiple venomous snakes, Kevlar chaps to prevent snake bites on the lower extremities. All personnel were misted down with 3% Lysol immediately upon exit of the cave. During necropsies, PPE was less cumbersome but included double latex gloves, disposable gowns, and powered air-purifying respirator (PAPR) units (3M).

To maximize the chances of isolating virus, large numbers of *R. aegyptiacus* were sampled over the course of four separate collections spanning one year and three months beginning in August 2008. Bats were captured and processed following procedures detailed in Towner et al. [10]. The notable exceptions to those procedures were that harp traps were used exclusively to

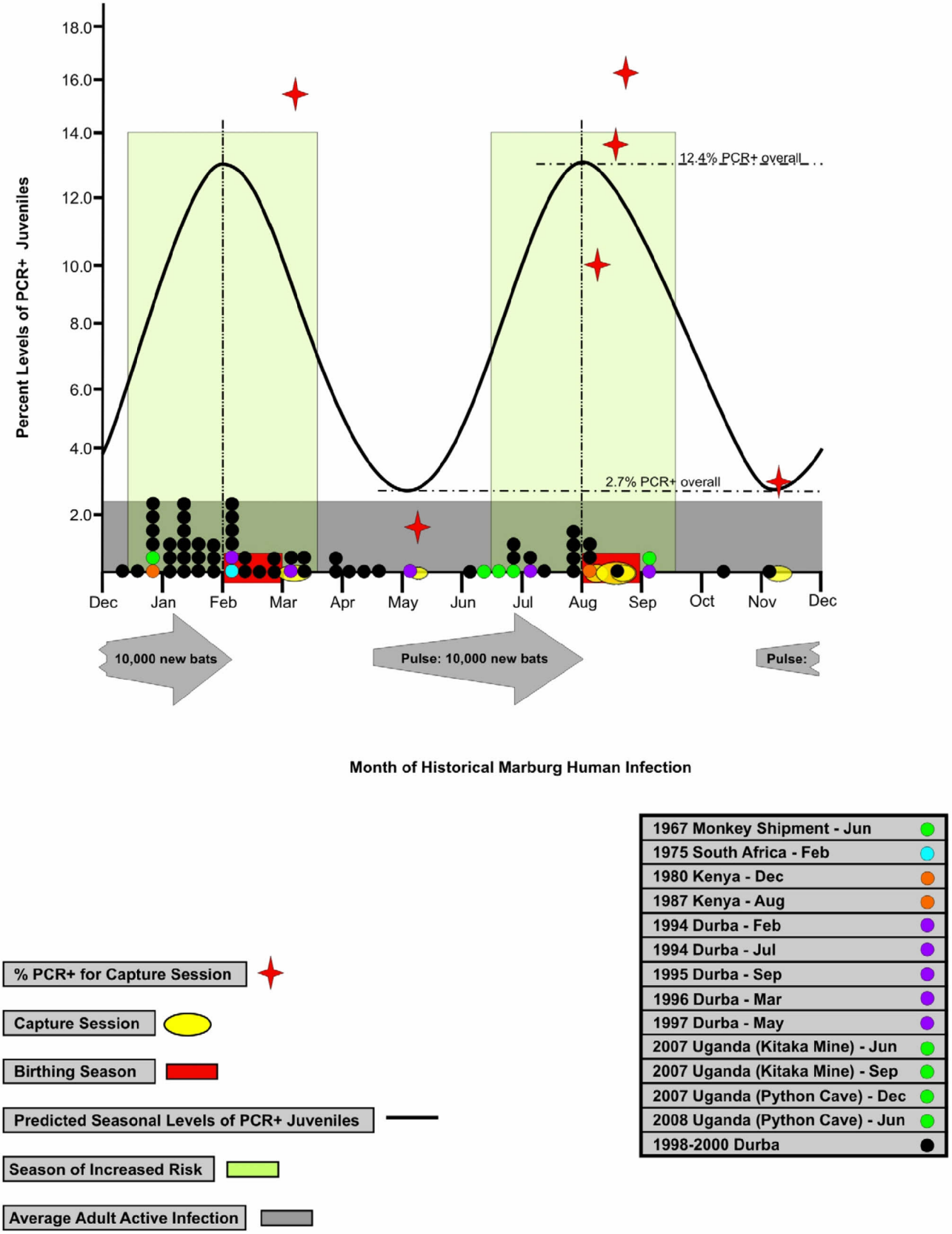

Figure 4. Increases in seasonal risk to human health. Historical spillover events (colored circles on X axis) compared to predicted seasonal levels of PCR+ juveniles (sinusoidal curve). The amplitude of the curve is based on average PCR+ juveniles experimentally determined during birthing (12.4%) and breeding (2.7%) seasons. Large light green vertical rectangles represent the proposed approximate three month seasons of increased risk

based on the average level of juvenile infected bats at peak times of encompassing birthing (February and August) and breeding (May and November). Large gray arrows depict the twice yearly influx of newly autonomous juvenile bats born in the prior birthing season. The influx begins at the approximate time of the juvenile's independence from their mothers.
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capture bats and more tissue types were collected. Replicate tissue samples were also preserved in 10% formalin for a minimum of four days and later changed to 70% ethanol for long term storage. Bats were identified morphometrically [24] and their measurements, sex, and breeding status were recorded

Collection of additional fauna

Adult and nymphal argasid ticks (14 pools of 10–20) were collected from crevices in the rocks near bat roosting sites and immediately placed in chaotropic RNA extraction buffer. Collections of endoparasites occurred during necropsies and were identified as tongue worms of the phylum Pentastomida. These parasites were typically found on the liver and spleen.

Virus isolation

Virus isolation attempts were carried out as described in Towner et al. [10]. Briefly, approximate 250 mg frozen tissue sections were placed on ice and homogenized in viral transport medium (HBSS/5% fetal calf serum) using sterile alundum (Fisher cat# A634-3) to form 10% suspensions. The homogenate was then spun at low speed for 5–10 minutes a 4°C and 100 ul of resulting supernatant was used to inoculate Vero E6 cells in 25 cm^2 flasks at 37°C/5% CO_2 for 1 hr. Media was then replaced with MEM/2% fetal calf serum and monitored for 14 days with a media change on day 7. All cultures were then tested by IFA for Marburg virus.

Q-RT-PCR, RT-PCR and nucleotide sequencing analysis

Q-RT-PCR, RT-PCR, and nucleotide sequencing, were all performed using reagents and procedures described in Towner et al. [10]. Briefly, virus inactivation in tissue samples was achieved by incubating approximate 100 mg of tissue samples from bats in 450 µl of 2X cellular cold lysis buffer (ABI) at 4°C for greater than eight hours. Each tissue was then diluted to 1X and homogenized for 2 minutes, at 1500 strokes/min using a ball-mill tissue grinder (Genogrinder 2000, Spex Centriprep). Total RNA was extracted from 150 ul of the homogenate [25] and tested for Marburg virus using slightly modified Q-RT-PCR [8] or nested RT-PCR assays. The Q-RT-PCR assay consisted of two reporter probes, 5′ Fam-ATCCTAAACAGGC"T"TGTCTTCTCTGGGACTT-3′ and 5′ Fam-ATCCTGAATAAGC"T"CGTCTTCTCTGGGACT-T-3′ in addition to the amplification primers (forward) 5′-GGACCACTGCTGGCCATATC-3′ and (reverse) 5′-GAGAA-CATITCGGCAGGAAG-3′. The quencher BHQ1 was placed internally in the probes at the "T" locations. The nested VP35 RT-PCR assay is previously described [6], and consisted of primers F1 (forward-outside) 5′-GCTTACTTAAATGAG-CATGG-3′, F3 (forward-inside) 5′- CAAATCTTTCAGCTA-AGG-3′, R1 (reverse-outside) 5′- AGIGCCCGIGTTTCACC-3′ and R2 (reverse-inside) 5′- TCAGATGAATAIACACAI AC-CCA-3′. The four primers used for the nested NP assay [9] are MBG704F1 (forward-outside) 5′-GTAAAYTTGGTGACAGGT-CATG-3′, MBG719F2 (forward-inside) 5′-GGTCATGATGCC-TATGACAGTATCAT, MBG1248R1 (reverse outside) 5′-CTCGTTTCTGGCTGAGG-3′, and MBG1230R2 (reverse inside) 5′-ACGGCIAGTGTCTGACTGTGTG-3′. The annealing conditions were 50°C for the first round (both assays) and 54°C (NP assay) or 50°C (VP35 assay) for the second round using high-fidelity one-step RT-PCR reagents (Invitrogen). Primer concentrations and amplification conditions used were as described by the manufacturer. Sequencing was performed using the appropriate amplification primers and standard di-deoxy sequencing methods.

Serology

Briefly, IgG detection was performed essentially as described in [26] with the exception that 96-well plates were coated with 200 ng/well of purified Marburg (Musoke) GP (Integrated BioTherapeutics, Gaithersburg, MD) or 200 ng/well of purified Ebola (Zaire) GP. The purified GPs contained a deletion of the trans-membrane domain (dTM) and were diluted in PBS. Bat sera were diluted 1:100 and four-fold through 1:6400 in 5% non-fat milk in PBS with 0.1% (vol/vol) Tween 20 (Bio-Rad Richmond, CA) and allowed to react with the GP-coated wells. Bound IgG was detected with goat anti-bat IgG (Bethyl cat# A140-118P) conjugated to horseradish peroxidase. Optical densities (OD) at 410 nm were recorded on a microplate spectrophotometer. The adjusted OD at 410 nm was generated by subtracting the OD of the well coated with Ebola-GP (dTM) from its corresponding Marburg GP-coated well. All sera were analyzed in duplicate and the threshold corrected ODs value for a positive Marburg IgG antibody test was determined to be 0.72 based on the mean corrected sum OD of the negative control group plus three standard deviations. The negative control group consisted of 210 young juvenile *R. aegyptiacus* (~three months old). This age group was chosen because they were the cohort considered least likely to have evidence of previous Marburg infection based on data presented here and previously [10] that suggest Marburg virus is transmitted horizontally and not vertically between bats.

Immunohistochemical analyses

Immunohistochemical analyses was performed following techniques described in [27] to determine if Marburg virus infection caused lesions in infected bats. Sections were cut from paraffin-embedded blocks prepared from formalin-fixed liver and spleen samples from 40 bats found positive by Q-RT-PCR, and examined concurrently with samples from 40 bats found negative by Q-RT-PCR. Hematoxylin and eosin (H&E) stained sections of the tissues were examined for lesions, and sections stained by an immune-alkaline phosphatase technique with a polyclonal rabbit anti-Marburg virus antiserum diluted to 1/1000.

Statistical analysis

All statistical analyses, Fisher's Exact and two-sided independent samples T tests, of the capture data were performed using PASW 18.0 (SPSS Statistics, Rel. 18.0.0. 2009. Chicago: SPSS Inc. an IBM Company).

Nucleotide sequencing and phylogenetic analysis

Sequencing of Marburg virus whole genomes and partial gene sequences (NP and VP35) were performed as previously described [8,9]. Multiple sequence alignments were generated in SeaView [28] using the MAFFT function [29]. A Bayesian phylogenetic analysis was conducted in MrBayes 3.2 [30] using the GTR+I+G model of nucleotide substitution. Two simultaneous analyses, each with four Markov chains, were run for 10,000,000 generations, sampling every 100 generations. Convergence was examined prior

to termination of the analysis by ensuring that the standard deviation of split frequencies had fallen below 0.01, thus confirming that the length of the run was sufficient. Trees generated before the stabilization of the likelihood scores were discarded (burnin = 100), and the remaining trees were used to construct a consensus tree. Nodal support was assessed by posterior probability values (≥.95 = statistical support). GenBank numbers for all sequences used in this study will be provided upon acceptance of this manuscript (see Table S2 for accession numbers).

Supporting Information

Table S1 Suspected (extrapolated) exposure dates for 52 miners from the final Marburg hemorrhagic fever (MHF) patient list from the 1998–2000 outbreak in Durba, Democratic Republic of Congo.
(DOCX)

Table S2 GenBank accession numbers of all Marburg virus sequences analyzed.
(DOCX)

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Author Contributions

Conceived and designed the experiments: BRA SAC PER STN JST. Performed the experiments: BRA SAC ZDR TKS SB RS AK BRE JAC SC DLC MLK PA CDP RJKC TDF KLW RU EKM RD JWT SRZ PER TGK STN JST. Analyzed the data: BRA SAC ZDR TKS RS AK BRE JAC SC DLC MLK CDP RJKC TDF SRZ PER TGK STN JST. Contributed reagents/materials/analysis tools: BRA SAC ZDR TKS SB RS AK JAC SC DLC MLK PA CDP RJKC TDF KLW RU EKM RD JWT SRZ PER TGK STN JST. Wrote the paper: BRA SAC PER STN JST.

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Perspective

The Next Epidemic — Lessons from Ebola

Bill Gates

Perhaps the only good news from the tragic Ebola epidemic in Guinea, Sierra Leone, and Liberia is that it may serve as a wake-up call: we must prepare for future epidemics of diseases that may spread more effectively than Ebola. There is a significant chance that an epidemic of a substantially more infectious disease will occur sometime in the next 20 years; after all, we saw major epidemics during the 20th century, including the Spanish influenza epidemic of 1918–1919 and the ongoing pandemic of human immunodeficiency virus. In fact, of all the things that could kill more than 10 million people around the world, the most likely is an epidemic stemming from either natural causes or bioterrorism.

Ebola is far from the most infectious known disease. Other disease agents (measles and influenza, for example) are far more infectious because they can be spread through the air, rather than requiring direct contact. People may not even be aware that they are infected or infectious. Since a person carrying one of these pathogens can infect many strangers in a marketplace or on an airplane, the number of cases can escalate very quickly.

As the Ebola epidemic fades from the world's attention, we risk missing the opportunity to learn from it. Even if the system we have today had worked perfectly for Ebola, it would fail to contain a more infectious disease.

It's instructive to compare our preparations for epidemics with our preparations for another sort of global threat — war. The North Atlantic Treaty Organization (NATO) has a mobile unit that is ready to deploy quickly. Although the system is not perfect, NATO countries participate in joint exercises in which they work out logistics such as how fuel and food will be provided, what language they will speak, and what radio frequencies will be used. Few, if any, such measures are in place for response to an epidemic. The world does not fund any organization to manage the broad set of coordinated activities required in an epidemic. The last serious simulation of an epidemic in the United States, the Dark Winter exercise, took place in 2001. And few countries have met their commitments under the International Health Regulations, which were adopted by the United Nations after the 2002–2003 outbreak of the severe acute respiratory syndrome (SARS) and were intended to improve the world's ability to prevent and contain outbreaks.[1]

Recommendations for Preparing for Future Epidemics

The world needs to build a warning and response system for outbreaks. This system should

- be coordinated by a global institution that is given enough authority and funding to be effective,
- enable fast decision making at a global level,
- expand investment in research and development and clarify regulatory pathways for developing new tools and approaches,
- improve early warning and detection systems, including scalable everyday systems that can be expanded during an epidemic,
- involve a reserve corps of trained personnel and volunteers,
- strengthen health systems in low- and middle-income countries, and
- incorporate preparedness exercises to identify the ways in which the response system needs to improve.

Because there was so little preparation, the world lost time in the current epidemic trying to answer basic questions about combating Ebola. In the next epidemic, such delays could result in a global disaster.

The problem is not the fault of any single institution — it reflects a global failure. The world needs a global warning and response system for outbreaks. (Though the World Health Organization [WHO] has a Global Outbreak Alert and Response Network, it is severely understaffed and underfunded.) Such a system could enable us to manage not only a naturally occurring epidemic, but also one ignited by a bioterror attack.[2] Although I have not seen a rigorous estimate of the cost of building such a system, World Bank projections give a sense of the cost of inaction: a worldwide influenza epidemic, for example, would reduce global wealth by an estimated $3 trillion.[3]

I hope the following sketch of what such a warning and response system might look like will spark action to prepare for an epidemic that could have global consequences (see box).

HEALTH SYSTEMS AND SURVEILLANCE

First, there is a critical need to reinforce basic public health systems, including primary health care facilities, laboratories, surveillance systems, and critical care facilities, among other components. As many commentators have noted, Ebola has spread much faster and more widely in countries whose health systems — and especially whose primary care systems — were severely weakened by years of armed conflict and neglect.

Strengthening health care systems not only improves our ability to deal with epidemics, but it also promotes health more broadly. Without a functioning health system, it is very hard for a country to end the cycle of disease and poverty. Health is so fundamental to development that even if there were no chance of another epidemic, building and improving health systems would be a worthwhile — and lifesaving — investment. The fact that they also bolster our ability to confront epidemics is all the more reason to invest in them.

In addition, there is no systematic disease-surveillance process in place today in most poor countries, which is where a naturally occurring epidemic seems most likely to break out. Even once the Ebola crisis was recognized last year, there weren't resources to effectively map where cases were occurring and in what quantity.

We need to invest in better disease-surveillance and laboratory-testing capacity, for normal situations and for epidemics. Routine surveillance systems should be designed in such a way that they can detect early signs of an outbreak beyond their sentinel sites and be quickly scaled up during epidemics. They should be linked with national public health laboratories to enable robust monitoring and response. And the data derived from such testing need to be made public immediately. Many laboratories in developing countries have been financed by the polio-eradication campaign, so we will have to determine what capacities will be needed once that campaign is over.

HUMAN AND OTHER RESOURCES

Once it became clear that a serious emergency was under way in West Africa, many local clinicians should have been recruited, and trained personnel should have flowed rapidly into the affected countries. That didn't happen. Some countries stepped forward with volunteers within 2 to 3 months, but they were needed within days. It was fortunate that Médecins sans Frontières could mobilize volunteers more quickly than any government.

We need trained personnel ready to confront and contain an epidemic quickly: incident managers; experts in epidemiology, disease surveillance, and other relevant fields who can provide surge capacity; respected community leaders who can lead local engagement efforts; and community workers who speak local languages. Ideally, we would have updated lists of such personnel indicating their availability and capabilities. There would also be standby training centers and an explicit understanding regarding compensation and insurance for volunteers. Each country could commit to managing a pool of volunteers and to sending a certain number of people with various skills and

equipment within a week after an emergency began, with plans for evacuating any who were exposed to the epidemic pathogen.

Transportation and equipment are also key. When an epidemic strikes, roads and airports in affected areas are overwhelmed by people trying to get out. Volunteers will be more likely to sign up if they know they will be able to leave if they get sick or when their duty is done. Few organizations are capable of moving thousands of people — some of them infected — to various locations around the world at a week's notice. The Ebola epidemic might have been much worse if the U.S. and U.K. governments had not used military resources to fly people in and out of the affected countries. All countries could identify trained military resources that would be available for epidemics; in a severe epidemic, the military forces of many or all middle- and high-income countries might have to work together.

During severe epidemics, responders also need tents, portable power sources, medical supplies, and other materials. A list of the supplies that would be needed to stop an epidemic affecting 10 million people — 100 times the population affected by the Ebola epidemic — could be developed, and experts could determine which items would need to be stockpiled or be subject to commandeering.

It is also critically important to have good data about what's going on. Unfortunately, during the Ebola epidemic, the case database has not always been accurate or up to date — partly because of the chaotic situation, but also because good technology and training have not been available and there are no clear rules regarding making data accessible. For future epidemics, it should be possible to have a system in which information on suspected cases, locations, survivors, and other key elements was entered into a digital database that was instantly accessible to the relevant organizations and agencies. The groups working on the Ebola data — including the WHO, the U.S. Centers for Disease Control and Prevention, and others — could recommend specifications, and some combination of foundations and technology companies could build such a system within the year.

Experts will also need computer models to predict what might happen and which interventions should be prioritized. With access to satellite photography and cell-phone data, they could understand the movement of populations and individuals in the affected region. But Internet and cell-phone capacity need to be improved. We should be able to use cell-phone systems to contact the public and to poll people about what they are seeing and experiencing. Key centers should have high-bandwidth Internet capacity through satellite, and Wi-Fi capacity should be added in key areas so that digital tools can help with reporting data and coordinating personnel.

MEDICAL AND PUBLIC HEALTH TOOLS

It should be possible to make diagnostic tests, drugs, and vaccine platforms that could be adapted for use against various pathogens. Today, with the possible exception of influenza vaccines, we do not have nearly enough capacity for developing adaptable platforms, partly because there are opportunity costs for private-sector organizations in shifting resources away from more commercially viable projects to work on tools for epidemics that may not happen. We may need an international funding system that factors in these opportunity costs.

Other than watching for symptoms, the diagnostic approach used during the Ebola epidemic has involved sending blood samples for quantitative polymerase-chain-reaction (qPCR) analysis. But qPCR machines are expensive and not widely available, so on average it has taken 1 to 3 days to get test results. For the next epidemic, an adequate number of qPCR machines should be made available while novel diagnostic methods are rapidly developed. We also need a clear process for developing and manufacturing accurate diagnostic tests rapidly. A focused effort to accelerate this process and establish a rapid approval and procurement process would be worthwhile.

On the therapeutics front, there are drugs that work against viruses similar to Ebola, and some of them have been shown in test assays to have an effect against Ebola. Unfortunately, they were not tested in patients with Ebola until after the epidemic had peaked — in part because there was no clear process for approving a novel trial format or for providing indemnity against legal liability. We will need to develop a clear set of guidelines (and testing and regulatory pathways) for determining whether existing drugs could be repurposed to help stop a particular epidemic.

We also need to invest in more research on antiviral drugs, antibody treatments, and RNA-based constructs. We should have either stockpiles or manufacturing capacity for therapies that might be effective in an epidemic.

Plasmapheresis should have been used in the Ebola epidemic,

but its application wasn't approved and scaled up until it was too late for this intervention to have a large impact. Plasmapheresis is quite effective for a number of diseases (including smallpox and viral hemorrhagic fevers such as Lassa fever) and has a reasonable chance of working for Ebola as well. The Gates Foundation started working to establish plasmapheresis units in early September 2014 and quickly found partners ready to take them into the affected countries. Unfortunately, the effort was hampered by the lack of a clear process for approving new approaches. We should develop rules now to expedite drug approvals in future epidemics and establish clear guidelines for approving studies and treatments, including experimental ones. A global epidemic-drug–approval process could avert long delays by indemnifying companies working on new approaches.

Three different Ebola vaccine constructs were being developed in the summer of 2014. Although all were in early stages, this work made us more prepared for Ebola than we would be for an entirely new pathogen, for which vaccine development could take 2 or more years. Moreover, it is not clear how quickly vaccine developers could or would move or who should finance the final research and manufacturing of a new vaccine.

Among known pathogens, influenza is the one most likely to cause a large epidemic; even seasonal influenza variants probably cause several hundred thousand excess deaths each year. So it's disappointing that we don't have a vaccine for all influenza strains. There is work being done toward this goal, but it has garnered nowhere near the resources that it deserves.

Ideally, vaccine research would be funded in such a way that during an outbreak, a vaccine could be designed, tested for safety, and ready for manufacture at scale within a few months. There is no guarantee of success, but I believe that given enough time and resources, such efforts could produce an invaluable contribution for epidemics and overall health.

Given Ebola's limited infectiousness in the early stages of the disease, most of the quarantine policies that were proposed would have been counterproductive. But when a far more infectious agent comes along, quarantine may be one of the few tactics that can reduce its spread in the early stages of disease. Because democratic countries try to avoid abridging individuals' rights to travel and free assembly, they might be too slow to restrict activities that help spread disease.

Part of the process should include a plan for effective public communications, including coordination of the messages conveyed by all the different voices people will hear, from governments, to United Nations agencies, to news media, to bloggers. Digital communication can be used to great advantage, but unless a plan is in place, it will only spread confusion and panic faster.

A GLOBAL CALL TO ACTION

Despite efforts by the United States and a few other countries, there are still big holes in the world's ability to respond to an epidemic. Other countries may be more likely to step up if they see an overall plan and understand their role in it. We need a rigorous study of the cost of building a global warning and response system and a plan for contributions from various countries.

Through the United Nations, some global institution could be empowered and funded to coordinate the system. The United Nations and the WHO are studying the lessons from the Ebola epidemic and ways to improve international crisis management; these evaluations can provide a starting point for discussions of ways to strengthen the WHO's capacity and about which parts of the process it should lead and which ones others (including the World Bank and the G7 countries) should lead in close coordination. The conversation should include military alliances such as NATO, which should make epidemic response a priority. The final arrangement should include a reserve corps of experts with the broad range of skills needed in an epidemic.

An epidemic is one of the few catastrophes that could set the world back drastically in the next few decades. By building a global warning and response system, we can prepare for it and prevent millions of deaths.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

A more detailed version of this article is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

From the Bill and Melinda Gates Foundation, Seattle.

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Avian Influenza Viruses in Water Birds, Africa

Nicolas Gaidet,* Tim Dodman,† Alexandre Caron,* Gilles Balança,* Stephanie Desvaux,* Flavie Goutard,* Giovanni Cattoli,‡ François Lamarque,§ Ward Hagemeijer,† and François Monicat*

We report the first large-scale surveillance of avian influenza viruses in water birds conducted in Africa. This study shows evidence of avian influenza viruses in wild birds, both Eurasian and Afro-tropical species, in several major wetlands of Africa.

Wild water birds are considered to be the major natural reservoir for avian influenza viruses (AIV) (*1*). Large numbers of Eurasian breeding water birds overwinter in the sub-Saharan region of the African continent (*2*), where the survival of AIV is considered to be restricted by the tropical environment (*3*). Although the first reported isolation of AIV from wild birds (A/Tern/S.A./61 [H5N3]) was in Africa (*4*), a knowledge gap exists in the ecology of AIV in tropical regions (*1,5*). Whether AIV circulate in waterbird communities in Africa and whether tropical ecosystems can play a role in the perpetuation of AIV among waterfowl remain unknown. We report results from large-scale surveillance of water birds in 12 countries in Africa (Figure).

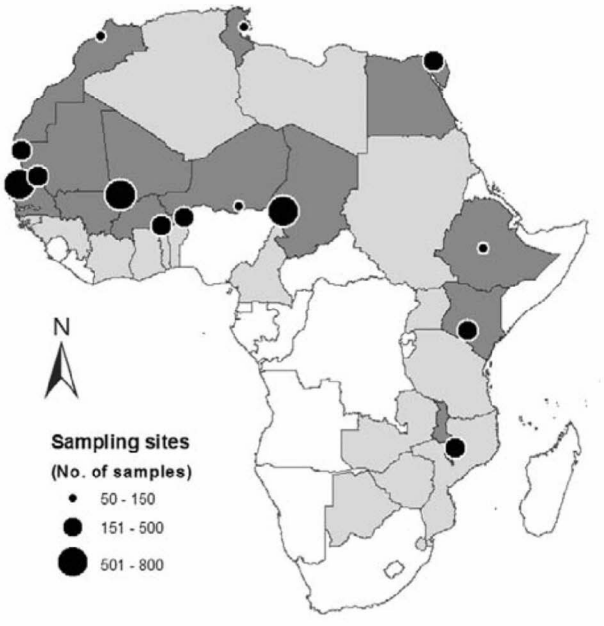

Figure. Locations of sampling sites (or clusters of sites) in surveyed African countries (dark gray) initially participating in the Food and Agriculture Organization's Technical Cooperation Programs (light and dark gray). All samples were collected from mid-January to early March 2006 (but until May in Tunisia).

The Study

This surveillance program was implemented in early 2006 within the framework of the Food and Agriculture Organization (FAO)'s Technical Cooperation Programs of Emergency Assistance for Early Detection and Prevention of Avian Influenza. Field sampling operations were coordinated by Centre de cooperation Internationale en Recherche Agronomique pour le Développement and by Wetlands International, in partnership with wildlife and veterinary national services, international organizations[1], local ornithologic nongovernment organizations, as well as national hunting associations and safari operators. Study species were selected among bird families recognized as major AIV reservoirs (notably among the orders Anseriformes and Charadriiformes), in both Eurasian and Afro-tropical bird communities. Study sites important for congregatory water birds were selected in accordance with national surveillance programs and field logistic constraints and included sites where palearctic and Afro-tropical birds mix.

From mid-January to early March 2006 (and early May in Tunisia), we collected cloacal swab samples from captured birds and from freshly killed birds provided by hunters. Samples of fresh droppings were also collected at roosting areas for gulls, terns, and some ducks. In Ethiopia, which has hunting restrictions, and in countries in which emergency surveillance operations were implemented after notification of influenza A (H5N1) outbreaks in Nigeria (Burkina Faso, Niger), special permits were obtained to shoot birds for sample collection (n = 732).

Materials used and storing procedures were standardized among field teams. The transport medium consisted

*Centre de Cooperation Internationale en Recherche Agronomique pour le Développement, Montpellier, France; †Wetlands International, Wageningen, the Netherlands; ‡Viale dell'Università, Legnaro, Italy; and §Office National de la Chasse et de la Faune Sauvage, Paris, France

[1]African Waterbird Ringing Scheme (AFRING), Oiseaux Migrateurs du Paléarctique Occidental (OMPO), Office National de la Chasse et de la Faune Sauvage (ONCFS), Dutch Centre for Field Ornithology or Stichting Openbaar Voortgezet Onderwijs Noord (SOVON), and Wildfowl and Wetlands Trust (WWT).

of an isotonic phosphate-buffered saline, pH 7.0–7.4, containing antimicrobial agents (penicillin 10,000 U/mL, streptomycin 10 mg/mL, amphotericin B 25 µg/mL, and gentamycin 250 µg/mL) supplemented with 10% glycerol. Samples were stored in liquid nitrogen containers or on ice and then stored at <–70°C after a few hours (generally <4 h, maximum of 24 h). They were shipped in dry ice in cryopacks until processed.

Samples were analyzed at the Istituto Zooprofilattico Sperimentale delle Venezie (Italy), except for samples from Egypt that were analyzed at the US Naval Medical Research Unit-3 (Egypt), samples from Kenya and Malawi which were analyzed at the Agricultural Research Council Onderstepoort Veterinary Institute (RSA), and samples from Tunisia which were analyzed at the Southeast Poultry Research Laboratory (USA). The samples were all screened by real-time reverse transcription (RT)–PCR specific for type A influenza viruses (*6*), and positive samples were tested by RT-PCR specific for H5 subtype. All type A–positive samples were subsequently processed for virus isolation by using standard methods (inoculation into the allantoic cavity of 9- to 10-day-old embryonated specific-pathogen–free eggs, EU directive 92/40). Isolates were characterized by hemagglutination and neuraminidase-inhibition tests by using specific hyperimmune chicken antisera to the reference strains of influenza virus (*7*). Molecular pathogenicity of H5 subtype–positive samples was determined by sequencing the hemagglutinin gene segment (BigDye Terminator v3.1 cycle sequencing kit, Applied Biosystems, Foster City, CA, USA).

A total of 4,553 birds (Table 1), consisting mostly of Afro-tropical and Eurasian ducks (32% and 31% of sam-

Table 1. Prevalence of avian Influenza virus in wild birds*

Bird group	Species tested	No.	PCR positive, no. (%)	Positive country
African ducks	9 species (total, including 4 named below)	1,455	41 (2.8)	
	Dendrocygna viduata	1,181	38 (3.2)	TD, ET, ML, MR, NE, SN
	Sarkidiornis melanotos	117	3 (2.6)	ML, NE
	D. bicolor	88	0	
	Plectropterus gambensis	32	0	
Eurasian ducks	10 species	1,409	93 (6.6)	
	Anas querquedula	1,335	87 (6.5)	TD, ML, MR, NE, SN
	A. acuta	24	2 (8.3)	ML
	A. crecca	24	3 (12.5)	MA
	A. clypeata	6	1 (16.7)	MA
Eurasian waders	13 species	409	6 (1.5)	
	Philomachus pugnax	115	2 (1.7)	ML
	Tringa glareola	74	0	
	Calidris minuta	60	0	
	C. ferruginea	45	2 (4.4)	TN
	Himantopus himantopus	45	0	
	Gallinago gallinago	30	0	
	T. erythropus	23	2 (8.7)	ML
Rails	8 species	438	3 (0.7)	
	Porphyrio alleni	187	0	
	Amaurornis flavirostris	88	0	
	Fulica cristata	80	0	
	Gallinula chloropus	31	2 (6.5)	ML
	Porphyrio porphyrio	10	1 (10)	ML
Gulls	3 species	366	14 (3.8)	
	Larus genei	156	13 (8.3)	SN
	L. fuscus	129	1 (0.8)	MR
	L. melanocephalus	81	0	
Terns	7 species	159	2 (1.3)	
	Sterna sp.†	150	2 (1.3)	MR
Cormorants	2 species	148	0	
	Phalacrocorax carbo	130	0	
Other	36 species	196	0	
Total	87 species	4,553	159 (3.5)	

*Detected by reverse transcription–PCR (RT-PCR), for all RT-PCR–positive species and in species with >30 individuals sampled. Lower numbers in individual species are included in the total for each bird group. Countries where RT-PCR–positive samples were obtained are indicated (TD, Chad; ET, Ethiopia; ML, Mali; MR, Mauritania; NE, Niger; SN, Senegal; MA, Morocco; TN, Tunisia).
†Unidentified fresh dropping samples from a multispecies flock of *Sterna caspia*, *S. maxima*, and *S. sandvicensis*.

ples, respectively), were tested. The overall protion of AIV detected was 3.5% (n = 159 RT-PCR–positive samples, including both cloacal swabs and fresh droppings). Low-pathogenicity AIV were detected in 14 species of ducks, waders, gulls, terns, and rails, including both Eurasian and Afro-tropical species (Table 1). Positive samples were obtained from 8 countries (Chad, Ethiopia, Mali, Mauritania, Morocco, Niger, Senegal, and Tunisia). In the 2 most frequently sampled species, Eurasian ducks (garganey [*Anas querquedula*], n = 1,329) and Afro-tropical duck (white-faced whistling ducks [*Dendrocygna viduata*], n = 1,157), AIV were detected from most surveyed countries but with a highly variable prevalence (Table 2). Neither influenza A (H5N1) viruses nor any highly pathogenic AIV were detected. A total of 11 samples were positive for H5 subtype, mostly from garganey ducks (H5 prevalence of 0.7%). Finally, 5 low-pathogenicity AIV were isolated: 3 distinct isolates that originated from garganey ducks sampled in the Inner Niger Delta in Mali (H5N3, H11N9, H12N5) and 2 isolates that originated from white-faced whistling ducks sampled in Ethiopia (H8N4) and Senegal (H1N1).

Conclusions

The African continent, in particular its sub-Saharan region, constitutes a seasonal shelter for a large number of Eurasian water birds, including an estimated 5.4 million ducks that gather in western and eastern Africa during the northern winter (*8*). In their overwintering sites, these birds congregate and mix with a wide variety of Afro-tropical water birds, some of them with large populations widespread over Africa.

AIV have been isolated in wild ducks on wintering grounds in both Europe and North America (*9,10*). Results from this surveillance program established that AIV are also present in wild birds in Africa during the northern winter. Low-pathogenicity AIV were detected and isolated in several species from several major wetlands of northern, western, and eastern Africa, which indicates that environmental conditions in Afro-tropical ecosystems are favorable for the persistence and transmission of AIV.

We detected and isolated AIV in Eurasian and Afro-tropical species. This finding shows that AIV circulate in migratory water birds originating from Eurasia and in African species that remain in the continent throughout the year. Moreover, the detection of viruses in some Eurasian wader species during wintering (in January in Mali) and during migration (in May in Tunisia) contrasts with the apparent absence of AIV reported from previous studies of waders in Europe (*5,11*). Since waders form the most abundant group of African-Eurasian migratory water birds (*12*), these shorebirds may play a role in maintaining some AIV in waterbird communities at wintering and stopover sites.

The detection of AIV in Eurasian ducks in several of their major overwintering sites in West Africa (e.g., the Inner Niger Delta, the Senegal River Delta, and Lake Chad) supports the hypothesis that AIV can persist in wild duck populations year-round through a continuous circulation in a proportion of birds (*1*). Variability in the prevalence observed might be related to differences in local logistical constrains but also to differences between African regions in their waterbird assemblage and connectivity with European breeding grounds. The different isolates obtained from garganey from the Inner Niger Delta also indicate that various subtypes are circulating at the same time in a population, a finding that agrees with patterns observed in Europe and North America (*11,13*).

Various AIV subtypes were isolated from apparently healthy garganey and white-faced whistling ducks, which indicates that both Eurasian and Afro-tropical ducks may serve as reservoirs of AIV. These results not only suggest that some Eurasian ducks could carry AIV on their northward spring migration but also raise the possibility that AIV could persist in the tropical region and be disseminated over Africa through intra-African migratory ducks. The presence of AIV at African wintering and stopover sites, where birds from various geographic origins congregate and mix, provides opportunities for transmission of AIV between different populations and spread of AIV over extensive areas in both Eurasia and Africa.

Table 2. Reverse transcription PCR–based detection of influenza A virus in 2 wild duck species sampled in different countries

Species	Country	No. samples tested	No. PCR positive (%)
Garganey (*Anas querquedula*)	Chad	381	11 (2.9)
	Kenya	104	0
	Mali	411	22 (5.4)
	Mauritania	225	33 (14.7)
	Niger	87	4 (4.6)
	Senegal	121	17 (14.0)
White-faced whistling duck (*Dendrocygna viduata*)	Burkina Faso	167	0
	Chad	232	1 (0.4)
	Ethiopia	76	10 (13.2)
	Malawi	59	0
	Mali	36	1 (2.8)
	Mauritania	183	7 (3.8)
	Niger	232	8 (3.4)
	Senegal	172	11 (6.4)

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Dr Gaidet is an ecologist in the Animal Production and Veterinary Department at the French Agricultural Research Centre for International Development. His primary research interests include the host ecology of avian influenza and West Nile viruses.

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Address for correspondence: Nicolas Gaidet, CIRAD, UR 22 Gestion Intégrée de la Faune, TA 30/E Campus International de Baillarguet, 34398 Montpellier, France; email: nicolas.gaidet@cirad.fr

Mopeia Virus–related Arenavirus in Natal Multimammate Mice, Morogoro, Tanzania

Stephan Günther, Guy Hoofd, Remi Charrel, Christina Röser, Beate Becker-Ziaja, Graham Lloyd, Christopher Sabuni, Ron Verhagen, Guido van der Groen, Jan Kennis, Abdul Katakweba, Robert Machang'u, Rhodes Makundi, and Herwig Leirs

A serosurvey involving 2,520 small mammals from Tanzania identified a hot spot of arenavirus circulation in Morogoro. Molecular screening detected a new arenavirus in Natal multimammate mice (*Mastomys natalensis*), Morogoro virus, related to Mopeia virus. Only a small percentage of mice carry Morogoro virus, although a large proportion shows specific antibodies.

Arenaviruses are segmented negative-strand RNA viruses. Their natural hosts are various rodent species. The virus family comprises several human pathogens causing hemorrhagic fever, namely Machupo, Guanarito, Junin, Sabia, and Chapare viruses in South America, and Lassa and Lujo viruses in Africa (*1–3*). In addition, Africa harbors arenaviruses that are not linked with human disease: Mobala, Ippy, Mopeia, and Kodoko viruses (*4–7*). We conducted a systematic search in wildlife in Tanzania to identify new African arenaviruses.

The Study

During 1985 through 1989, a total of 2,520 small mammals were live-trapped in different regions of Tanzania. After species determination, they were measured and bled by orbital puncture. Serum samples were tested by indirect immunofluorescent antibody (IFA) assay (*8*). Lassa virus was used as antigen due to its cross-reactivity with immune sera from animals infected with other arenaviruses (*4,6*). Clusters of seropositivity were found in *Arvicanthis* spp. rodents from the Iringa region (20%) and in Natal multimammate mice (*Mastomys natalensis*) from Arusha (18%) and Morogoro (17%) (Table 1), which suggests that these animals are reservoirs of arenaviruses. Titers ranged from 16 to 512 and 16 to 4,096 in *Arvicanthis* spp. rodents and *M. natalensis* mice, respectively. Peak prevalence in *M. natalensis* mice was found on the campus of the Sokoine University in Morogoro (23.7% of 746 animals collected over several seasons).

In 2004, *M. natalensis* mice were trapped in a mosaic of maize fields and fallow grassland at the university campus in the city of Morogoro (6°50′34.9794″S; 37°38′8.232″E) to identify the virus. The animal voucher specimens were deposited at the Royal Museum of Central Africa, Tervuren, Belgium. RNA was prepared from 10 μL of rodent serum by using the QIAamp Viral RNA kit (QIAGEN, Valencia, CA, USA), and screening was performed by using a pan–Old World arenavirus reverse transcription–PCR (RT-PCR) specific for the large (L) gene (*9*). One of 96 serum samples was positive (no. 3017/2004) (Table 2), and sequencing of the PCR fragment showed a new arenavirus sequence. The virus was isolated in Vero cells and called Morogoro virus (strain 3017/2004).

For sequencing, the isolate was propagated in T75 flasks, virus particles in supernatant were pelleted by ultracentrifugation, and RNA was isolated by using the QIAamp Viral RNA kit (QIAGEN). The entire 3.5-kb small (S) RNA segment was amplified by RT-PCR as described previously (*10*). The 7-kb L RNA segment was amplified in 2 fragments by using a long-range RT-PCR protocol and primers targeting the conserved termini of L RNA and Morogoro virus–specific primers designed on the basis of the sequence of the fragment detected by RT-PCR screening. By using the PCR products as a template, short overlapping fragments were amplified and sequenced with a set of consensus primers for Old World arenaviruses, and S and L RNA sequences were assembled (GenBank accession nos. EU914103 and EU914104). (Sequences reported in this article have been submitted to GenBank and assigned the following accession numbers: full-length S and L RNA sequences of Morogoro virus, EU914103–04; partial L gene sequences of Morogoro virus, EU914107–22; cytochrome B gene of Morogoro virus-positive *Mastomys natalensis*, EU914105–06.)

Full-length amino acid sequences of glycoprotein precursor (GPC), nucleoprotein (NP), and L protein of Morogoro virus were aligned with published Old World arenavirus sequences and pairwise p distances were calculated. Morogoro virus showed genetic similarity to strains

Author affiliations: Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany (S. Günther, B. Becker-Ziaja); Institute of Tropical Medicine Leopold II, Antwerp, Belgium (G. Hoofd, G. van der Groen); Université de la Méditerranée, Marseille, France (R. Charrel); Artus Company, Hamburg (C. Röser); Centre for Emergency Preparedness and Response, Salisbury, UK (G. Lloyd); Sokoine University of Agriculture, Morogoro, Tanzania (C. Sabuni, A. Katakweba, R. Machang'u, R. Makundi) University of Antwerp Department of Biology, Antwerp (R. Verhagen, J. Kennis, H. Leirs); and University of Aarhus Department of Integrated Pest Management, Kongens Lyngby, Denmark (H. Leirs)

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Table 1. Detection of African arenavirus-specific antibodies in small mammals in Tanzania, 1985–1989*

Genus	Antibody detection† by region (no. positive/no. tested)									Total
	Arusha	**Iringa**	**Lindi**	Mbeya	**Morogoro**	Mtwara	Ruvuma	Songea	Tanga	
Acomys	–	0/3	0/2	0/2	0/57	0/2	–	–	–	0/66
Aethomys	–	0/3	0/4	–	0/23	0/11	0/7	0/8	–	0/56
Arvicanthis	0/13	**6/30**	–	–	–	–	–	–	0/87	**6/130**
Cricetomys	–	–	–	–	0/35	–	–	–	–	0/35
Lemniscomys	0/5	**1/2**	**1/2**	–	**1/30**	0/2	0/1	–	–	**3/42**
Lophuromys	0/3	0/1	–	–	0/3	–	–	–	0/7	0/14
Mastomys	**7/39**	0/17	**1/120**	0/12	**181/1,054‡**	0/81	0/8	0/25	0/82	**189/1,438**
Mus	–	0/1	–	0/1	**1/47**	–	–	–	–	**1/49**
Praomys	–	0/3	–	0/1	0/1	–	–	–	0/1	0/6
Rattus	–	–	0/24	0/1	0/49	0/20	0/3	0/15	0/196	0/308
Tatera	0/1	0/1	0/32	–	0/127	0/69	0/11	0/3	–	0/244
Uranomys	–	–	–	–	0/11	–	–	–	–	0/11
Sciuridae	–	–	0/13	–	0/2	–	0/2	–	0/10	0/27
Crocidura	–	–	–	–	**1/14**	–	–	–	–	**1/14**
Petrodomus	–	–	0/9	–	–	0/18	–	–	–	0/27
13 other genera	–	0/1	0/2	0/7	0/21	0/20	–	–	0/2	0/53
Total	**7/61**	**7/62**	**2/208**	0/24	**184/1,474**	0/223	0/32	0/51	0/385	**200/2,520**

*Positive samples as well as the respective sampling sites and animals are indicated in **boldface**.
†Immunofluorescent antibody (IFA) assay was performed with Lassa virus–infected cells (cut-off titer 16).
‡Fifty IFA assay–positive serum samples were randomly selected and tested by immunoblotting. Presence of African arenavirus–specific antibodies, as defined by reactivity with Lassa virus nucleoprotein and glycoprotein 2, was confirmed in 47 serum specimens.

of Mopeia virus that were circulating in Mozambique (*4*) and Zimbabwe (*5*). A close relationship between both viruses was also demonstrated by phylogenetic analysis using GPC, NP, and L gene sequences (Figure 1, panel D, and data not shown). Both viruses are sister taxa, sharing a common ancestor with Mobala virus.

Although the distances between Morogoro and Mopeia virus in the amino acid sequence of GPC (12%), NP (12%–13%), and L gene (26%) were higher than intraspecies differences among known African arenaviruses (i.e., pairwise differences between strains of the same species; <11% in GPC and NP; <21% in L), they did not reach the level of interspecies distances (>20% in GPC and NP; >37% in L) (Figure 1, panels A–C). Therefore, we currently consider Morogoro virus a subspecies of Mopeia virus rather than a new arenavirus species. This classification is supported by the fact that both viruses share the same host. Sequencing of the mitochondrial cytochrome b gene of rodent liver samples positive for Morogoro virus confirmed that its natural host is *M. natalensis* mice (GenBank accession nos. EU914105 and EU914106).

An additional 303 ethanol-preserved liver samples and 63 serum samples were collected in 2004 and 2007, respectively. Liver tissue (≈3 mg) was homogenized by using a bead mill. Cell debris was pelleted by centrifugation, and RNA was isolated from the homogenate with the RNeasy Mini kit (QIAGEN). Testing by L gene RT-PCR (*9*) showed 16 positive liver and serum samples, which indicated a virus prevalence in the *M. natalensis* population of ≈4% (Table 2). PCR fragments were sequenced (GenBank accession nos. EU914107–EU914122), and Morogoro virus was isolated in cell culture from all 4 PCR-positive serum samples obtained in 2007. Morogoro virus–specific antibodies in serum samples from 2004 and 2007 were measured by IFA assay using Vero cells infected with Morogoro virus. The antibody prevalence was ≈50%, which compares quite well with the 23% prevalence determined in this area 20 years before. In some animals, virus and antibodies were detected (Table 2).

The availability of Morogoro virus L gene sequences from 2004 and 2007, originating from the same host population (trapping sites <1 km apart), provided us with the

Table 2. Prevalence of Morogoro virus and Morogoro virus–specific antibodies in *Mastomys natalensis* mice from Morogoro University campus, Tanzania

Specimen and year of sampling	No. samples	No. (%) virus positive (PCR)	No. (%) antibody positive*	No. (%) antibody plus virus positive
Serum 2004	96	1 (1)†	42 (44)	0
Liver 2004	303	12 (4)†	–	–
Serum 2007	63	4 (6)‡	40 (63)	3 (5)§

*By immunofluorescent antibody (IFA) assay, performed with Morogoro virus-infected cells (cut-off 32).
†Testing was performed with universal Old World arenavirus large (L) gene reverse transcription–PCR (*9*).
‡Testing was performed with Morogoro virus–specific L gene RT-PCR using primers MoroL3359-forward (5′-AGGATTAGTGAGAGAGAGAGTAATTC-3′) and MoroL3753-reverse (5′-ACATCATTGGGCCCCACTTACTATGGTC-3′).
§Titers ranged from 64 to 512.

opportunity to estimate the molecular clock rate for this virus. Phylogenetic reconstruction was performed with the BEAST version 1.4.8 package (http://beast.bio.ed.ac.uk) (*11*) under the assumption of a relaxed lognormal molecular clock and general time reversible (GTR) or Hasegawa-Kishino-Yano (HKY) substitution model with gamma-distributed substitution rate variation among sites (Figure 2 and data not shown). Analysis was run for 2 million Markov chain Monte Carlo steps, which yielded a reliable set of data as verified with the TRACER program (http://tree.bio.ed.ac.uk/software/tracer). Based on GTR and HYK model, 3.2×10^{-3} and 3.4×10^{-3} substitutions per site and year (95% interval of highest posterior density $1.1–6.6 \times 10^{-3}$), respectively, were calculated.

Conclusions

A serologic survey in small mammals from Tanzania identified a hot spot of arenavirus circulation in Morogoro

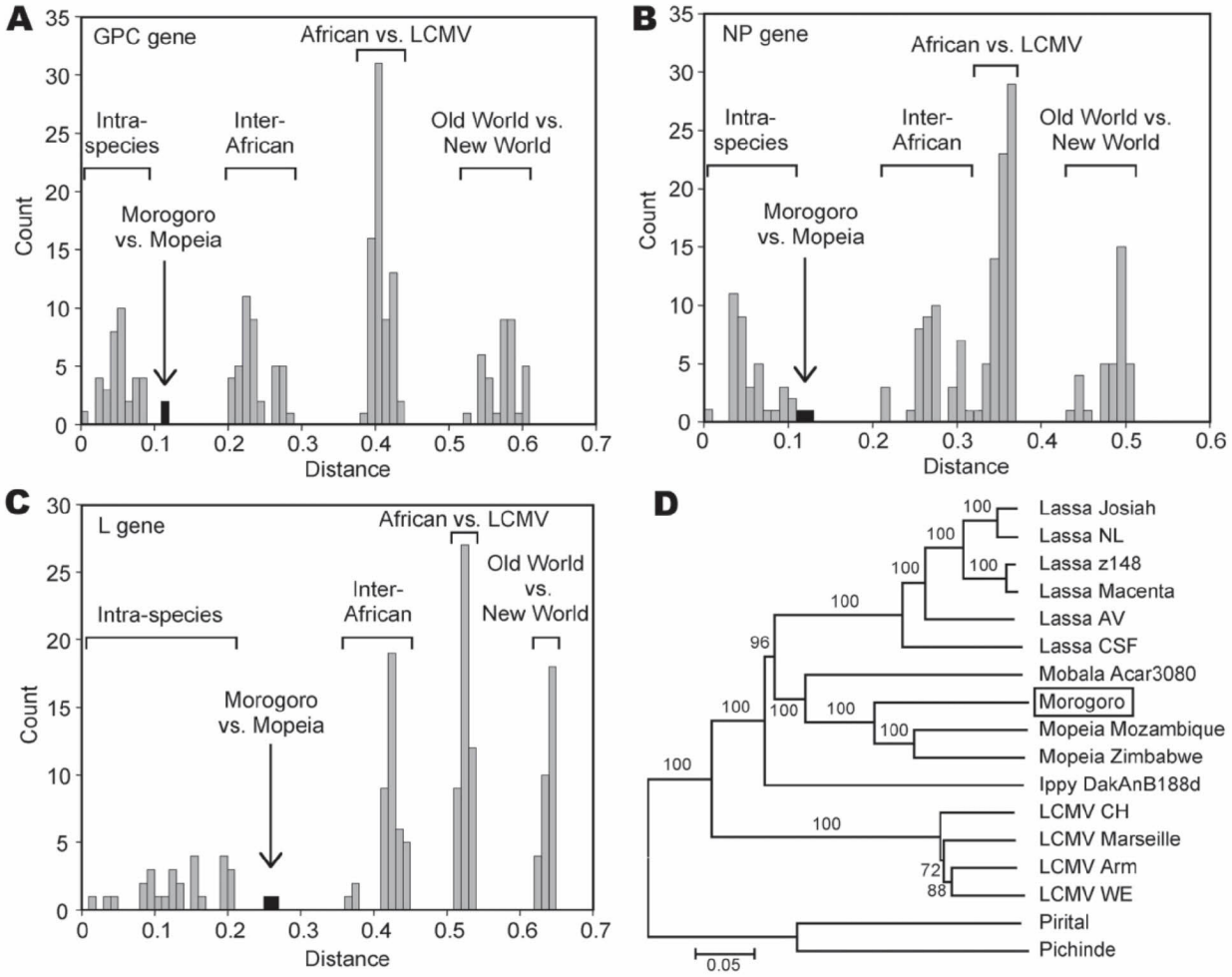

Figure 1. Genetic distances and phylogenetic relationship among arenaviruses, including Morogoro virus. Amino acid sequence diversity was calculated using p distance. Full-length glycoprotein precursor (GPC), nucleoprotein (NP), and large (L) gene amino acid sequences of the following arenaviruses were pairwise compared: Lassa virus (strains Josiah, NL, Z148, Macenta, AV, and CSF), Mobala Acar3080, Morogoro 3017/2004, Mopeia virus (strains Mozambique and Zimbabwe), Ippy DakAnB188d, lymphocytic choriomeningitis virus (LCMV) (strains CH-5692, Marseille, Armstrong, and WE for all genes; Traub and Pasteur for GPC and NP only), Pirital, and Pichinde. Frequency histograms of pairwise distances are shown for A) GPC gene; B) NP gene; and C) L gene. The ranges for intraspecies distances (i.e., pairwise differences between strains of the same virus species); distances between different African arenavirus species; between African arenaviruses and LCMV; and between Old World and New World viruses are marked above the bars. Bars representing the distances between Morogoro virus and the most closely related viruses (Mopeia virus strains) are filled in black. D) Phylogeny of Old World arenaviruses based on full-length L gene amino acid sequences. The tree was inferred by using the neighbor-joining method implemented in the MEGA software package (www.megasoftware.net). The New World arenaviruses Pirital and Pichinde were used as outgroups. Numbers represent bootstrap support (1,000 replications). Identical trees with respect to the phylogenetic position of Morogoro virus (shown in the box) were obtained with full-length GPC and NP amino acid sequences (not shown). Scale bar indicates nucleotide substitutions per site.

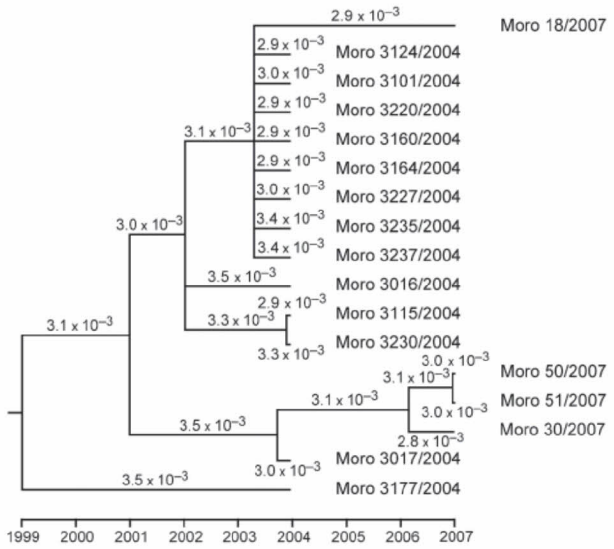

Figure 2. Phylogenetic tree and molecular clock of Morogoro virus based on partial large gene sequences of 17 strains (340 nucleotides; GenBank accession nos. EU914104 and EU914107–EU914122). Phylogeny was inferred with the BEAST v1.4.8 package (*11*) under assumption of a relaxed lognormal molecular clock and general time reversible substitution model with gamma-distributed substitution rate variation among sites. Branches with posterior probability <0.5 were collapsed. The substitution rate per site and year is indicated for each branch. Node ages and rates are median values. Variation in rates among branches is low as calculated with Tracer program (beast.bio.ed.ac.uk/Tracer) indicating a molecular clock in the evolution of Morogoro virus. The same tree topology with similar substitution rates was obtained when assuming the Hasegawa-Kishino-Yano substitution model (not shown).

in the late 1980s. This work is being published now because early attempts to substantiate the existence of the virus failed. The identification of the virus was facilitated by a recently developed pan–Old World arenavirus PCR (*9*) that also led to the discovery of new arenaviruses in rodents from West Africa (*7*). Only a small percentage of *M. natalensis* mice carry Morogoro virus, and a large proportion shows specific antibodies, which indicates that most animals clear the virus during life. Viruses and antibodies, which are presumbably directed to nucleocapsid proteins, also co-circulate, as seen in hantavirus infection in rodents (*12*). Detection of Morogoro virus in the liver is consistent with the organ tropism of Lassa virus in *M. natalensis* mice (*13*). In agreement with studies on Lassa virus strains, the largest genetic distance between Morogoro and Mopeia virus was seen in L gene, which contains several highly variable regions (*14*).

The clock rate estimate of 3×10^{-3} for Morogoro virus L gene is in agreement with that of other RNA viruses (*15*), although it must be interpreted with caution, given that the difference in date between the samples is not large. The tree topology did not correlate with geographic or ecologic sampling data.

The pathogenicity of Morogoro virus for humans is not known, though its phylogenetic clustering with African arenaviruses that are not linked with human disease (*4–6*) and the absence of hemorrhagic fever in the area suggest that it does not cause severe disease. Hospital-based investigations are required to estimate the public health relevance of this virus.

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Dr Günther is head of the Virology Department at the Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany. His research interests are molecular biology and epidemiology of arenaviruses, in particular, Lassa virus.

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Address for correspondence: Stephan Günther, Bernhard-Nocht-Institute for Tropical Medicine Department of Virology, Bernhard-Nocht-Str 74, 20359 Hamburg, Germany; email: guenther@bni.uni-hamburg.de

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Continent-wide panmixia of an African fruit bat facilitates transmission of potentially zoonotic viruses

Alison J. Peel[1,2], **David R. Sargan**[1], **Kate S. Baker**[1,2,3], **David T.S. Hayman**[1,2,4,5,6], **Jennifer A. Barr**[7], **Gary Crameri**[7], **Richard Suu-Ire**[8,9], **Christopher C. Broder**[10], **Tiziana Lembo**[11], **Lin-Fa Wang**[7,12], **Anthony R. Fooks**[5,13], **Stephen J. Rossiter**[14], **James L.N. Wood**[#1], and **Andrew A. Cunningham**[#2]

[1]Department of Veterinary Medicine, University of Cambridge, Cambridge, CB3 0ES, UK

[2]Institute of Zoology, Zoological Society of London, Regent's Park, London, NW1 4RY, UK

[3]Wellcome Trust Sanger Institute, A1301, Hinxton, Cambridgeshire, CB101SA, UK

[4]Wildlife Zoonoses and Vector-Borne Diseases Research Group, Department of Virology, Animal Health and Veterinary Laboratories Agency, Weybridge, New Haw, Addlestone, Surrey, KT15 3NB, UK

[5]Department of Biology, Colorado State University, Fort Collins, Colorado, CO 80523, USA

[6]Department of Biology, University of Florida, Gainesville, FL 32611, USA

[7]CSIRO Australian Animal Health Laboratory, Geelong, Victoria, 3220, Australia

[8]Wildlife Division, Ghana Forestry Commission, Accra, Ghana

[9]University of Ghana, Faculty of Animal Biology and Conservation Science, Box LG 571, Legon, Accra, Ghana

[10]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, Maryland, 20814-4799, USA

[11]Boyd Orr Centre for Population and Ecosystem Health, Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, U.K.

[12]Duke-NUS Graduate Medical School, Singapore 169857

[13]University of Clinical Infection, Microbiology and Immunology, Liverpool, L3 5TQ, UK

[14]School of Biological and Chemical Sciences, Queen Mary University of London, London, E1 4NS, UK

[#] These authors contributed equally to this work.

Corresponding authors: James L.N. Wood, Department of Veterinary Medicine, University of Cambridge, Cambridge, CB3 0ES, UK. Ph: +44 (1223) 764 666, jlnw2@cam.ac.uk; Andrew A. Cunningham, Institute of Zoology, Zoological Society of London, Regent's Park, London, NW1 4RY, UK. Ph: +44 207 449 6674, A.Cunningham@ioz.ac.uk.

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Abstract

The straw-coloured fruit bat, *Eidolon helvum*, is Africa's most widely distributed and commonly hunted fruit bat, often living in close proximity to human populations. This species has been identified as a reservoir of potentially zoonotic viruses, but uncertainties remain regarding viral transmission dynamics and mechanisms of persistence. Here we combine genetic and serological analyses of populations across Africa, to determine the extent of epidemiological connectivity among *E. helvum* populations. Multiple markers reveal panmixia across the continental range, at a greater geographical scale than previously recorded for any other mammal, whereas populations on remote islands were genetically distinct. Multiple serological assays reveal antibodies to henipaviruses and Lagos bat virus in all locations, including small isolated island populations, indicating that factors other than population size and connectivity may be responsible for viral persistence. Our findings have potentially important public health implications, and highlight a need to avoid disturbances which may precipitate viral spillover.

Introduction

Recent studies have demonstrated the potential of bats to act as reservoirs of zoonotic pathogens (reviewed in [1]). One example is the common and conspicuous straw-coloured fruit bat (*Eidolon helvum*), which has been identified as a reservoir host for Lagos bat virus (LBV, family Rhabdoviridae, genus *Lyssavirus*)[2] and henipaviruses (family Paramyxoviridae)[3] in mainland Africa. *E. helvum* is a gregarious, predominantly tree-roosting species and large roosts (sometimes numbering more than one million bats) frequently exist in close proximity to large human settlements, including Accra (Ghana), Abidjan (Côte d'Ivoire), Dar es Salaam (Tanzania), Lagos (Nigeria), and Kampala (Uganda)[4].

Much of the serological evidence for zoonotic pathogens in bats comes from single cross-sectional studies, with few conducted longitudinally or across a representative proportion of the entire species range. However, longitudinal surveys of *E. helvum* colonies in Ghana have demonstrated relatively high roost-level seroprevalences to LBV over multiple years, which increase with bat age[5]. These findings indicate endemic circulation with horizontal transmission, making *E. helvum* a true reservoir host of LBV in that country. Moreover, neutralising antibodies to LBV have also been detected in cross-sectional serological surveys in Kenya[6] and Nigeria[7] and LBV has been isolated from a small number of sick or dead wild *E. helvum* bats in Nigeria, Senegal and Kenya (reviewed in [2]).

Old World fruit bats (Pteropodidae) are the principal reservoir hosts of henipaviruses [8], with flying fox populations (*Pteropus* spp.) found to harbour Nipah virus (NiV) in Southeast Asia, and both Hendra virus (HeV) and Cedar virus (CedPV) in Australia. NiV and HeV are highly pathogenic in humans and other mammals, yet the recently discovered CedPV differs in its apparent apathogenicity in laboratory animal species[9]. Cross-neutralising antibodies to HeV and NiV have been detected in sympatric *Pteropus* spp. and Madagascan fruit bats (*E. dupreanum*) [10], and Hayman et al.[3] first documented antibodies to henipaviruses in bats outside of the range of *Pteropus* spp, with a 40% seroprevalence being found in *E. helvum* in Ghana. These serological findings were recently supported by the detection of henipavirus-like RNA in *E. helvum* in Ghana and central Africa[11-13]; yet, while a full genome sequence for one of these African henipavirus-like viruses was obtained[13], live viruses have not yet been isolated.

These findings collectively highlight the potential for zoonotic pathogen spillover from *E. helvum* to humans, with routes of infection being via urine[12], faeces[13] or the hunting and preparation of bat meat for food[14]. However, no such spillovers have been reported for LBV

or African henipaviruses. This might be because spillover has not yet occurred, or it might reflect poor medical surveillance capabilities in much of Africa, and the lack of availability of specific diagnostic assays[15].

Much is yet to be understood regarding the host response to natural lyssavirus and henipavirus infections in bats; experimental inoculations have yielded inconsistent results across individuals and studies. Bats infected with lyssaviruses may or may not develop clinical signs corresponding to those seen in other mammals (reviewed in [2]), whereas no clinical illness has been observed in bats infected with henipaviruses[8]. Acute antibody responses have been observed for both viruses after experimental infection, with boosted titres upon reinfection[8,16]. An assumption could follow that these infections are immunising in bats, however seroconversion is not universally observed, and therefore this remains open to challenge. Typically, pathogens causing acute immunising infections require large host population sizes and a 'critical community size' (CCS) for persistence is expected unless birth rates are very high.

Many uncertainties also remain regarding the specific viral transmission dynamics in *E. helvum*. Key aspects of this species' ecology might further increase potential for viral persistence within populations. In particular, it is a migratory species that comprises both permanent and seasonal colonies across much of sub-Saharan Africa[4] and a small number of offshore islands, including those in the Gulf of Guinea[17] (Fig 1). However, the widespread and continuous distribution represented in Fig 1 over-simplifies a more intricate distribution pattern, comprising aggregated populations across a connected, rather than continuous, landscape[18]. Annual seasonal migrations result in abrupt fluctuations in the size of permanent colonies, and also in the formation of solely seasonal colonies. For example, the largest known *E. helvum* colony in Kasanka National Park in Central Zambia is populated rapidly each year to reach an estimated 1.5 million individuals [19], and persists for just 2 ½ months. Satellite telemetry studies indicate that these bats are capable of migrating vast distances (e.g. up to 370km in one night and ~2500km over 5 months)[20]. It has been suggested that migration occurs along a 'north-south' axis, with seasonal movements following latitudinal shifts of the Inter-Tropical Convergence Zone weather system[20,21]; however the routes and drivers of migrations are not fully understood. Such large-scale movements are expected to lead to widespread gene flow, and it has been argued that extensive genetic mixing among wildlife populations may increase the potential for viral epidemics[22]. Therefore, to characterise viral infection dynamics in wildlife populations, information on host population structure and connectivity is needed. Indeed, Plowright et al.[23] suggested that a large, weakly-coupled asynchronous metapopulation structure might be necessary for population-level persistence of HeV, with either acute 'explosive', or slow 'smouldering' epidemics resulting from spatial heterogeneity in population herd immunity. We recently demonstrated evidence of exposure to henipaviruses in the small, isolated population of *E. helvum* on the Gulf of Guinea island of Annobón, indicating that a metapopulation model may not be required for persistence of all henipaviruses[24]. The persistence of lyssaviruses in some temperate insectivorous bat species has been shown to depend on certain life history traits, including hibernation and birth pulses[25], but persistence mechanisms in non-hibernating species, such as *E. helvum*, are unknown.

To determine the extent of genetic and epidemiological connectivity among *E. helvum* populations, and thus gain better understanding of viral transmission dynamics and zoonotic risk, here we combine genetic and serological analyses of populations across Africa. We use mitochondrial (mtDNA) and nuclear DNA analyses to characterise the range-wide metapopulation structure of *E. helvum*, and hypothesise that this would inform our understanding of viral dynamics across the population. Together with serological analyses,

we assess the epidemiological consequences of this structure for the species' ability to act as a reservoir host of the potentially zoonotic viruses, LBV and henipaviruses.

Results

Sampling

Samples (including wing membrane biopsies, blood and urine) were obtained from 2,013 individual *E. helvum* bats across continental Africa and the Gulf of Guinea islands. Additionally, pooled urine samples were collected from beneath some colonies. Details of sampling locations (Fig 1 and Supplementary Data 1), sample sizes for genetic, serological and urine analyses (Table 1) are provided.

Microsatellite and Mitochondrial DNA Genetic analyses

Overall, results from multiple analyses presented below showed that *E. helvum* forms a panmictic population across its continental range, with no evidence of isolation by distance (IBD) or structuring according to migratory routes. The offshore island of Bioko was found to be part of this panmictic population; however, the more isolated island populations in the Gulf of Guinea were genetically distinct from one another and from the continental population.

Of 114 unique cytochrome *b* (cytb) haplotypes identified from 544 individuals, 75% were singletons (only found in a single individual across all populations, Table 2). Haplotype diversity, molecular diversity, allelic richness and observed heterozygosity were all higher within continental with Bioko (CB) populations than in isolated island (iIS) populations. Nucleotide diversity was low across all populations, but particularly so in Príncipe and Annobón.

Structure among populations assessed by pairwise F_{ST} (using microsatellite data) and ϕ_{ST} (using mtDNA data) values gave similar results, with near-zero, non-significant values among CB populations, contrasting with larger, significant values between iIS and CB populations (Supplementary Table S1). Each island population was also significantly differentiated from one another. These results were supported by analysis of molecular variance (AMOVA), where maximal structure among groups (high F_{CT} and ϕ_{CT} values) and minimal structure among populations within groups (low F_{SC} and ϕ_{SC}), were observed when populations were separated into three (CB, São Tomé with Príncipe (STP), Annobón) or four (CB, São Tomé, Príncipe, Annobón) groups (Table 3, analyses 7 and 8). Isolation by distance analyses detected no positive correlation between genetic distance (Slatkin's linearised ϕ_{ST} and F_{ST}) and log geographical distance in any mtDNA or microsatellite analyses (Fig. 2). This finding was consistent when latitude was ignored and longitudinal distances were used in the analyses, accounting for presumed north-south migration routes of *E. helvum*[21].

A Bayesian phylogeny (Supplementary Fig. S1) and median joining haplotype network (Fig 3) both recovered three main *E. helvum* clades. The star-like network was characterised by a few common haplotypes, surrounded by many haplotypes present in only 1–5 individuals. Thorough spatial mixing was evident, with the central haplotype (Hap2) being shared by 85 bats representing all CB populations plus a single bat from Annobón. Most bats from the isolated island (iIS) populations (253/272; 93%) were divided between two haplotypes at opposite ends of the network (Hap8, predominantly Annobón, and Hap111, predominantly STP; Supplementary Fig. S2).

Consistent with these results, Bayesian clustering of individual genotypes revealed three clusters (K=3) based on mean likelihood (log P (X|K) values (Fig 4), corresponding to

populations from CB, STP, and Annobón. With increasing values of K, the STP and Annobón clusters remained unchanged, and the CB cluster became increasingly subdivided into multiple clusters of approximately equal proportion (Fig 3), again indicative of a strong signature of a single panmictic CB population. Analyses run with CB or iIS samples as separate datasets did not reveal additional clusters. Using these three clusters as prior population information to identify potential migrants among clusters, STRUCTURE assignment tests (admixture analyses based on nuclear data), indicated that 19/502 individuals were 'admixed' (*i.e.* had an assignment probability (p) to any one main cluster of $0.8 > p > 0.2$). No bats were classified as recent (first generation) migrants (Supplementary Table S2).

Isolation-with-migration models and approximate Bayesian computation were unsuccessful in obtaining reliable estimates of gene flow between these islands, as a result of lack of convergence or unrealistically large estimates of effective population size, respectively.

Lagos bat virus serological analyses

Using modified Fluorescent Antibody Virus Neutralisation (mFAVN) assays, neutralising antibodies to LBV were detected in all continental and island locations (Table 1), yet seroprevalences showed significant variation by geographical location. A strikingly low LBV seroprevalence relative to other locations was observed in the Annobón population ($\chi^2 = 66.5$, $p < 0.001$), but seroprevalences in Bioko, São Tomé and Príncipe were not significantly different from mainland populations. Excluding Annobón and populations with sample sizes that were insufficient to allow a reliable seroprevalence to be calculated (Malawi, Zambia and Uganda; n = 12, 9 and 4, respectively), the mean LBV seroprevalence was 34% (95% CI: 32–37%) and the range of adult seroprevalences was 24–51% (Supplementary Data 1). In the Annobón population, neutralising antibodies to LBV were detected in 1 of 72 (1.4%, 0.0–7.5%) bats sampled in 2010 [24], and in 6 of 49 (14%, 7–27%) bats sampled in 2011.

Henipavirus serological analyses

Antibodies binding to NiV soluble G (sG) glycoproteins were detected using Luminex® microsphere binding assays in all populations sampled (Table 1). In contrast to the LBV results, henipavirus seroprevalences in all Gulf of Guinea islands (including Annobón) were similar to those in continental populations. Excluding populations with very small sample sizes, as above, the mean henipavirus seroprevalence was 42% (39–44%), with adult seroprevalences ranging from 29–60% (Supplementary Data 1). Using virus neutralisation tests (VNTs), a NiV seroprevalence of 5% (11/222, 3–9%) was detected in bats sampled from Tanzania and 1.7% (2/118, 0.5–6%) in bats from Annobón. For bats from Bioko, São Tomé, and Príncipe, NiV VNTs were performed on a subset of the samples (those with binding assay median fluorescence intensities (MFIs) > 750 (n = 49, 20 and 39, respectively)), of which 32%, 50%, and 51% were neutralising, respectively.

For both LBV and henipaviruses, no significant differences in seroprevalence were detected between males and females.

Urine analyses

PCRs performed on *E. helvum* urine samples from Tanzania, Uganda, Malawi, Zambia and Annobón detected paramyxovirus polymerase gene sequences in 3/23 extraction pools (from Ugandan and Tanzanian sampling sites, Table 1). These showed close relationships with sequences detected previously in *E. helvum* in Ghana [12] (Fig. 5). One PCR-positive pooled sample from Tanzania comprised urine expressed directly from the bladders of 6 individual

E. helvum, all of which were seronegative for henipaviruses using microsphere binding assays and VNTs.

Discussion

In this study, using data from both mtDNA and microsatellite markers, we demonstrate that the population of *E. helvum* is panmictic across its continental African range. An absence of IBD indicated that gene flow was no more likely to occur among neighbouring populations than distant populations of > 4,500 km, making *E. helvum* the largest reported panmictic unit of any mammal, and one of the largest of any vertebrate, exceeded only by the bigeye tuna (*Thunnus obesus*; > 8000 km)[26,27] and the Kentish plover (*Chadadrius alexandrines*; > 10,000km)[28]. Even present day human populations retain genetic structure over such large distances[29]. In fact, the range of *E. helvum* extends further north and west of the sampling sites in this study, so additional sampling is required to assess whether panmixia extends across this range; a distance of > 6,500 km.

The hypothesis that greater genetic differentiation might exist across migratory pathways (on an east-west axis) than along migratory pathways (on a north-south axis) was not supported by our results, probably either because gene flow between distinct migratory populations homogenises allele frequencies, or because *E. helvum* migration is opportunistic and tracks changes in available food resources rather than following defined migratory routes.

Included in the panmictic *E. helvum* population are bats on the near-shore island of Bioko in the Gulf of Guinea (which separated from the African continent ~7000 years ago). Our results indicate that the 32 km stretch of ocean that separates Bioko from the continent is not a significant barrier to dispersal, as might be expected given that individuals are capable of covering such distances during foraging bouts[20].

In contrast to the panmictic continental and Bioko (CB) population, populations on the three more isolated Gulf of Guinea islands (São Tomé, Príncipe and Annobón) showed evidence of genetic isolation. This accords with results from studies of other Gulf of Guinea island taxa, including other species of bat[30], bird (e.g. [31]), and reptile (e.g. [32]). Although *E. helvum* is a long-range migrant and has been observed as a vagrant on islands 570km from the African coastline[33], the strong genetic structure detected among the island and CB population clusters and the absence of genetic evidence (using assignment tests) of recent migrants between these clusters, indicate that dispersal between clusters (with successful mating) is likely to be rare: no first generation migrants were detected, although some individuals may have been second or third generation migrants. Additional support for population genetic results in this and other fruit bat species comes from genetic studies of external parasites and their pathogens [34,35], including detection of congruence between population genetic structure of external parasites and their hosts.

The Gulf of Guinea ocean channels are likely to have provided a barrier to initial colonisation and inter-island dispersal. Also, we found that the smallest discrete population of *E. helvum* (on the island of Annobón) showed genetic divergence and is truly isolated. Our mtDNA and microsatellite results are consistent with those of a previous study that found that *E. helvum* on Annobón showed differences in morphological traits and allozyme frequencies compared to other islands[17]. However, while Juste et al.[17] also concluded that a lack of phenetic differentiation on Bioko, São Tomé and Príncipe suggested gene flow among the islands, our use of multiple nuclear and mtDNA markers, provides further insight. For example, while São Tomé shows greater connectivity with Príncipe (< 150 km apart) than with either the CB or the Annobón populations (which lie >220 km away), the

distance separating these two populations is still a substantial barrier to inter-island gene flow, as shown by significant pairwise Φ_{ST} and F_{ST} values. Since São Tomé and Príncipe are within the same cluster, it is not possible to identify migrants between these two islands using assignment tests. Other genetic methods to estimate gene flow and demographic history between multiple populations, including isolation-with-migration models and approximate Bayesian computation, were unsuccessful in obtaining reliable and credible estimates of gene flow between these islands, suggesting that even for our substantial datasets, modelling of low rates of gene flow using current techniques and assumptions is not robust.

While genetic analyses cannot replace direct studies on individual bat movements and demographic connectivity, they can contribute to a broader perspective upon which to base epidemiological studies on transmission and maintenance of viruses among and within populations[36]. The strong genetic clustering observed here makes it likely that the separation of *E. helvum* into three distinct genetic population clusters (CB, STP & AN) is echoed as at least three epidemiologically-distinct populations. A freely mixing, panmictic continental population would likely facilitate viral transmission among *E. helvum* colonies across this range. Our serology results are consistent with this, with henipavirus and LBV antibodies being detected across all continental sampling sites at seroprevalences similar to those previously observed for henipaviruses in Ghana [3] and for LBV in Ghana, Kenya and Nigeria[5-7,37].

Further support for the conclusion that distant continental populations may belong to a single epidemiological unit was provided by high nucleotide sequence identities between paramyxoviral sequences detected in *E. helvum* urine samples from Uganda and Tanzania and those already reported from Ghana[12]. In that study and others[13,38], a diverse range of paramyxovirus sequences, including henipavirus-like sequences, were detected within single *E. helvum* populations. Further sampling efforts to enable exploration of viral sequence diversity across all the sites studied here would help determine whether different virus variants are maintained by each of these distinct epidemiological units and whether viral diversity may play a role in within-population viral persistence. Additional data are required to fully understand how virus variants are maintained within *E. helvum* populations.

Although genetic differentiation and isolation of *E. helvum* in the STP cluster was expected to be reflected epidemiologically, perhaps with an absence of antibodies on these islands due to restricted population sizes, we found that seroprevalences to both viruses were comparable to those on the mainland. These data suggest that: population sizes on each island are sufficient to maintain LBV and henipaviruses and are above the critical community size (CCS) required for persistence (although this concept requires further theoretical exploration for animal populations where birth rates, and hence population sizes, are highly seasonal); sufficient movement may occur between the two islands to maintain a larger epidemiologically connected population; alternative hosts may be involved; or our original assumptions on transmission and persistence may need re-examination (see below). The use of satellite telemetry has been enlightening in other fruit bat species [39,40] and would be required to definitively assess movement patterns of bats on these two islands. However, dispersal between São Tomé and Príncipe was suggested by our observation of a single asynchronous birth on Príncipe in the absence of other pregnant or lactating females, but which was contemporaneous with the presence of neonates on São Tomé. The asynchronous Príncipe birth is highly unusual for a species which employs delayed implantation to facilitate a highly synchronised birth pulse. If the two populations are connected via dispersal, the asynchrony in reproductive seasons between São Tomé and Príncipe could facilitate viral persistence by staggered introduction of susceptible individuals, via birth, into the population. Finally, LBV has been detected in bats of several species in Africa, with

ranges overlapping that of *E. helvum*[2], but the role that inter-species transmission plays in the maintenance of LBV in its host populations remains a gap in our knowledge. Of all these species, only *Rousettus aegyptiacus*, the Egyptian fruit bat, is present on São Tomé and Príncipe. This is a cave-roosting species, and mixed colonies with *E. helvum* are unlikely although these two species might mix at feeding sites. LBV has been isolated from *R. aegyptiacus* on two occasions (reviewed in [41]), and seroprevalence levels comparable to those reported in *E. helvum* were detected in Kenya[6]. On São Tomé and Príncipe, *R. aegyptiacus*, or indeed other species, may facilitate the persistence of LBV in *E. helvum*.

While findings from the CB and STP populations could be consistent with a metapopulation model of persistence, as proposed for HeV in Australia[23] and NiV in Malaysia [42], our results from Annobón indicate that this appears unnecessary for the persistence of henipaviruses or LBV in *E. helvum*. On Annobón, *E. helvum* is the only bat species confirmed to be currently present and has a population size of only ~2,500[24]. Surprisingly, and in contrast to findings in other, less-isolated island systems[42], the henipavirus seroprevalence in the Annobón *E. helvum* population was within the range of that observed in both the CB and the STP populations. Conversely, the Annobón LBV seroprevalence was much lower than in other populations. While evidence of infection with lyssaviruses has been reported in other island bat species (e.g. [43,44]), the bat populations in those studies were either much larger, within flight distance of continental bat populations, and/or hosted multiple sympatric bat species. In Annobón, all LBV seropositive individuals were adult, and further longitudinal studies are required to determine whether LBV is persistently maintained on this island (i.e. the population size is greater than the CCS), or whether these findings represent a single epidemic wave subsequent to introduction of the virus from another population. Unfortunately, deriving a quantitative estimate for the CCS is problematic, particularly for virus-host systems where little information is available regarding host demographics, virus-transmission mechanisms and within-host immune responses[45]. For both LBV and henipaviruses, important areas of future study include viral diversity and phylogeography, within-host persistence and immunity, incubation periods, and frequency- vs density-dependent transmission.

Multiple henipavirus-like sequences have been previously reported in *E. helvum*[11-13]. In the absence of isolation or full genomic characterisation, it cannot be definitely confirmed whether these sequences represent true henipaviruses. However, a phylogenetic analysis undertaken here, incorporating the most recently isolated henipavirus (CedPV in Australia) and sequence fragments from bat paramyxoviruses worldwide (Fig. 6) demonstrates that two virus sequences from *E. helvum* in Gabon[13] fall within the clade of currently-identified henipaviruses. These sequences therefore likely represent true African henipaviruses.

This study took a multidisciplinary approach, combining ecological, genetic and serological studies, to explore the ways in which the structure, dynamics and connectivity of *E. helvum* populations across Africa affects the viral transmission dynamics within them. These critical population-level processes are expected to be important in determining viral persistence within populations, and yet, while the three genetically-distinct populations identified here are also highly likely to be separated epidemiologically, each of these population clusters is capable of maintaining henipaviruses and LBV, apparently without the need for a metapopulation model of persistence via migration and reintroduction.

The findings presented here have potentially important implications for public health. The large population sizes of *E. helvum*, its tendency to roost and feed in close proximity to human populations, its extensive distribution across Africa and its frequent harvesting for bushmeat, present numerous opportunities for the exposure of people to excreta, tissues and body fluids from these bats. The widespread presence of potentially zoonotic viruses in this

species across Africa might therefore be of significant public health concern. Despite the possibility for undiagnosed spillover, the lack of detection makes it unlikely that pathogenic henipaviruses from *E. helvum* are regularly crossing the species barrier and undergoing significant sustained transmission in humans at this point in time. Spillover of NiV into pig populations in Malaysia may have occurred at least once prior to detection of the major outbreak[46], and therefore, detection of henipavirus antibodies in pigs in Ghana[47] warrants further study. Although no human cases of LBV infection have been reported, this virus causes clinical rabies in other mammalian hosts[2], and may not be detected as a cause of human rabies unless specific molecular-based LBV assays are performed.

Changes in bat-human interactions and bat-domestic animal interactions are hypothesised to be a catalyst for the zoonotic spillover of novel viruses from wildlife. Stressors, such as habitat loss, land-use change and increasing bat-human interactions may precipitate viral spillover from bats to other species[23]. Understanding viral persistence and the potential for spillover in African bat populations in the face of extensive hunting, logging, and human population growth is of central importance for both public health and conservation, especially since these processes can be expected to increase over time.

Methods

Sampling

All fieldwork was undertaken under permits granted by national and local authorities, with ethical approval from the Zoological Society of London Ethics Committee (project reference WLE/0489). Personal protective equipment (long clothing, face masks, eye protection and gloves) was worn during sample collection. Sampling was conducted in geographically widespread E. helvum populations along longitudinal and latitudinal axes across the species' range (Fig 1, Supplementary Data 1). In São Tomé, bats were obtained in collaboration with local hunters, who hunted at roost sites during the day or feeding sites at night. Elsewhere, bats were captured at the roost with mist nets (6-18m; 38mm) as they departed the roost site at dusk, or returned at dawn.

Female reproductive status was assigned as non-reproductive, pregnant, or lactating, assessed visually or via abdominal palpation. Age was assessed by morphological characteristics and all individuals could be allocated into one of four age classes: Neonate (<2mths), Juvenile (J; 2 – <6 months), Sexually Immature (SI; 6 – <24 months) or Adult (A; ≥24 months). For a subset of samples, the timing of sampling allowed further classification of SI individuals into 6-month age groups SI.1, SI.2 and SI.3 (6 <12, 12 – <18, 18 – <24 months, respectively).

Genetic and blood samples were collected under manual restraint. Wing membrane biopsies (4-mm) were placed into 70% alcohol. Up to 1 ml blood was collected from the propatagial vein using a citrated 1ml syringe and placed into a plain 1.5ml eppendorf tube. Pooled urine samples (up to 500 μl) were collected by pipette from plastic sheeting placed under E. helvum colonies in Tanzania and Uganda at dawn [12], or directly from individual bats (in Tanzania, Malawi, Zambia and Annobón), and frozen at −80°C without preservative. 'Populations' were initially defined arbitrarily based on national borders related to roost location.

Molecular methods

Genomic DNA was extracted from *E. helvum* tissues using DNeasy Blood and Tissue Kits (QIAGEN Ltd., Crawley, West Sussex, UK) and was supplied for one *E. dupreanum* bat from Madagascar by the Institut Pasteur de Madagascar. Multiplexed genotyping was performed using 18 loci in six multiplexed reactions (TSY, FWB, MNQX, AgPK, AcAfAi,

AdAh) using a Type-it Multiplex PCR Master Mix (QIAGEN Ltd.). From twenty *E. helvum* loci developed in a previous study[48], Loci E and Ae were discarded due to difficulty in scoring or high error rates and data were locus Ag were re-binned and re-scored, correcting earlier issues with allelic dropout. Positive and negative controls were included on each plate. Amplification of mtDNA cytb gene fragments from continental samples used generic primers L14722 (5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G)[49] and H15149 (5′- AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A)[50] in 20 μl reactions, containing 0.1–1ng template DNA, 0.2 μM of each primer, 0.25mM of each dNTP, 1.5mM $MgCl_2$, 0.25 μl of *Taq* polymerase (Invitrogen), and 0.2 μl 10× reaction buffer and with the following conditions: 5 min at 94°C; 40 cycles of 1 min at 93°C, 1 min at 54°C, and 2 min at 72°C; then 7 min at 72°C. Although these generic primers were adequate with continental samples (8% PCR failure), amplification from isolated Gulf of Guinea island samples was less successful (48% PCR failure). Shortened primers (EhM2814 (5′-GCT TGA TAT GAA AAA CCA TCG TTG) and EhM2815 (5′-CAG CCC CTC AGA ATG ATA TTT GT) resulted in successful amplification when using Microzone MegaMix-Gold reagent (Microzone Ltd, UK). PCRs were performed in 20 μl reactions, containing 2ng template DNA, 0.25 μM of each primer, and 10 μl MegaMix-Gold, using the following conditions: 5 min at 95°C; 33 cycles of 30 sec at 95°C, 30 sec at 53°C, and 45 sec at 72°C. PCR products were sequenced in both directions, aligned, manually checked and trimmed to 397 bp. No sequence differences were detected in 38 samples sequenced using both primer pairs, so data were combined.

RNA was extracted from urine samples using the MagMAX viral RNA isolation kit (Life Technologies, Paisley, UK), and the presence of paramyxovirus polymerase gene RNA was tested for using two heminested RT-PCRs (*PAR-F2*: GTT GCT TCA ATG GTT CAR GGN GAY AA, *PAR-R*: GCT GAA GTT ACI GGI TCI CCD ATR TTN C) [12,51].

Genetic Data Analyses

After removing non-independent samples (known or suspected offspring of other individuals within the dataset), cytb analyses and microsatellite analyses (at 17 loci) were performed on data from 544 and 502 individuals, respectively (Table 1). Abbreviations for population groupings used in analyses are CT (all continental populations), CB (all continental populations plus Bioko), IS (all four island populations), iIS (three isolated island populations (São Tomé, Príncipe and Annobón)) and STP (São Tomé and Príncipe) (Supplementary Fig. S3).

The statistical power of the microsatellite and mtDNA datasets to reject a null hypothesis of genetic homogeneity was assessed using the software POWSIM [52]. Values from the empirical datasets (number of populations, population sample sizes, number of loci and allele frequencies) were used to simulate 1,000 random sets of 12 subpopulations with expected F_{ST} values of 0.001 – 0.01. Since mtDNA is haploid, the sample size was halved for mtDNA analyses. Power calculations indicated that the inability to detect population structure among CB populations was not as a result of insufficient power within the dataset, and the estimated probability of falsely detecting significant differentiation was in line with the typically-accepted 0.05 cutoff. Analyses were therefore continued as described below.

For microsatellite data, departures from Hardy Weinberg Equilibrium (HWE) and the presence of linkage disequilibrium (LD) among loci were assessed using FSTAT v2.9 [53] and GENEPOP v4.0.10 [54], respectively. Significance levels were adjusted for multiple testing using the false discovery rate (FDR) method. Genetic diversity for each population and region was assessed by calculating observed heterozygosity (H_O), expected heterozygosity (H_E), and average allelic richness (R_S) in FSTAT. Population structure was assessed by calculating pairwise F_{ST} values between populations and by analysis of molecular variance

(AMOVA), as implemented in the software ARLEQUIN v3.5 [55]. Significance levels were obtained with 10,000 permutations. Data were tested for presence of isolation by distance by regressing natural logarithm-transformed geographical distances between sampling sites (in km) against Slatkin's linearised F_{ST} (F_{ST}/(1-F_{ST})). Statistical significance was assessed using a Mantel test with 10,000 permutations in ARLEQUIN.

Bayesian clustering analyses were performed 20 times for each value of K (K=1 to 13, representing the number of populations) for 1.5×10^6 iterations with 500,000 burn-in steps using the admixture model with correlated allele frequencies in STRUCTURE [56]. Analyses were repeated for separate continental and island datasets. Symmetric similarity coefficients (SSC) were used to assess consistency among replicate runs for each value of K using the Greedy algorithm of CLUMPP v1.1 [57], and only runs with SSC > 0.8 were included in further analyses. Individual membership coefficients from replicate runs were visualised graphically using the software DISTRUCT v1.1 [58]. To ensure that some loci not in HWE in the Bioko populations (see results) were not affecting clustering from this population, analyses were repeated separately with data from loci in or out of HWE in Bioko. No difference was seen in the results, and therefore remaining analyses were run with 16 loci. Assignment tests were performed in STRUCTURE and admixture was assessed using the USEPOPINFO option, using the clustering partition with the optimal mean log likelihood value as prior population information. Based on their assignment probability, p, individuals were considered non-migrant ($p > 0.8$), admixed ($0.2 > p > 0.8$), or a recent migrant ($p < 0.2$) [59]. STRUCTURE and CLUMPP analyses were performed using the CamGrid distributed computing resource. Comparable analyses were performed using spatially explicit methods, however the results were consistent and are not presented here.

For mtDNA, in addition to AMOVA and IBD analyses, descriptive parameters of genetic diversity were calculated in the software DnaSP v5.10 [60]. Rarefaction down to the minimum sample size was used to calculate haplotypic richness (HR, a measure of diversity standardised across population sample sizes) using the software RAREFAC [61]. Pairwise Φ_{ST} values were calculated in ARLEQUIN and significance values were adjusted for multiple comparisons using the FDR method. Median joining networks (MJNs) were constructed in the software NETWORK v4.6 [62]. For comparison, statistical parsimony networks were constructed using TCS [63], with a 95% parsimony connection limit, however the results were consistent and are not presented here. A phylogeny of unique cytb haplotypes was reconstructed by Bayesian inference in MRBAYES v3.1.2 [64], using the *E. dupreanum* cytb sequence as an outgroup (which was found to be 91% (360/397 bp) identical to the consensus *E. helvum* cytb sequence). The most appropriate substitution model (GTR + I) was selected using PAUP* v4.0b10 [65] and MODELTEST v3.7 [66]. MRBAYES was run with 4 simultaneous chains, sampled every 100 generations, and the first 25% of trees were discarded as burn-in. Generations were added until the standard deviation of split frequencies was below 0.015 (10×10^6 generations).

The relative contributions of isolation and gene flow (migration) on observed levels of population divergence were estimated using an isolation-with-migration model in IMa2 [67]. Once priors had been optimised, analyses were run until stationarity was reached, which took ~2–3 months and 1.7 – 46 million steps, depending on sample size, before genealogy sampling commenced. Genealogy information was saved every 100 steps, and sampling was continued until ~100,000 genealogies were available for each pairwise comparison (~ 1 month, depending on sample size). Eight competing colonisation scenarios were explored by analysing microsatellite and mtDNA data using Approximate Bayesian Computation (ABC) methods in the software DIYABC v 1.0 [68]. Eight different colonisation scenarios were considered.

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To construct a phylogenetic analysis of known henipaviruses and henipavirus-like viruses globally and other known Paramyxovirinae, sequences of a 559 bp segment of the polymerase gene were obtained from GenBank (Supplementary Table S3). Phylogenetic trees from these sequences and of viral sequences from urine samples analysed in this study were constructed using MRBAYES under the GTR+I+G model.

Serological analyses

The number of samples analysed using various serological assays for HeV, NiV and LBV is shown in Table 1. Antibodies against LBV (LBV.NIG56-RV1) were detected using a mFAVN assay [37], using the LBVNig56 isolate. Samples were tested in duplicate using threefold serial dilutions and titres corresponding to 100% neutralisation of virus input are reported as IC100 endpoint reciprocal dilutions and were considered positive at > 1:9.

Antibodies against henipaviruses (HeV and NiV) were detected using Luminex® multiplexed microsphere binding assays and VNTs using purified recombinant expressed henipavirus sG glycoproteins[69], which were conjugated to internally coloured and distinguishable microspheres, allowing multiplexing. Antibody binding to each microsphere was detected after conjugation of bound antibodies with biotinylated Protein A and fluorescent streptavidin-R-phycoerythrin. Binding results are given as MFI values of at least 100 microspheres for each virus type, and an MFI > 500 was considered positive[70]. Alternative, lower cutoffs were also considered based on results from mixture model analyses[70]. These resulted in higher seroprevalences, but no overall change in patterns to the higher, more conservative, cutoff presented here. In VNTs, samples exhibiting virus neutralisation at dilutions of ≥ 1:10 were considered positive. Stronger results were consistently observed in NiV binding assays and VNTs[24], so only NiV results are reported here. Chi-squared tests were used to detect significant ($p < 0.05$) variations in seroprevalences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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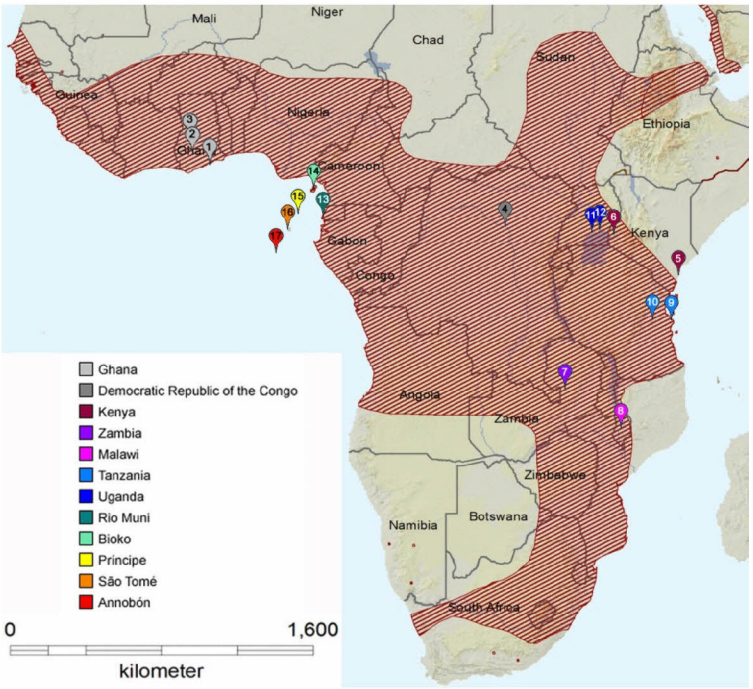

Fig 1. Map showing location of *E. helvum* sampling locations for genetic and serological analyses
Shading represents the distribution range of *E. helvum.* Sampling locations are numbered as in Supplementary Data 1. Adapted from Mickleburgh et al. [4].

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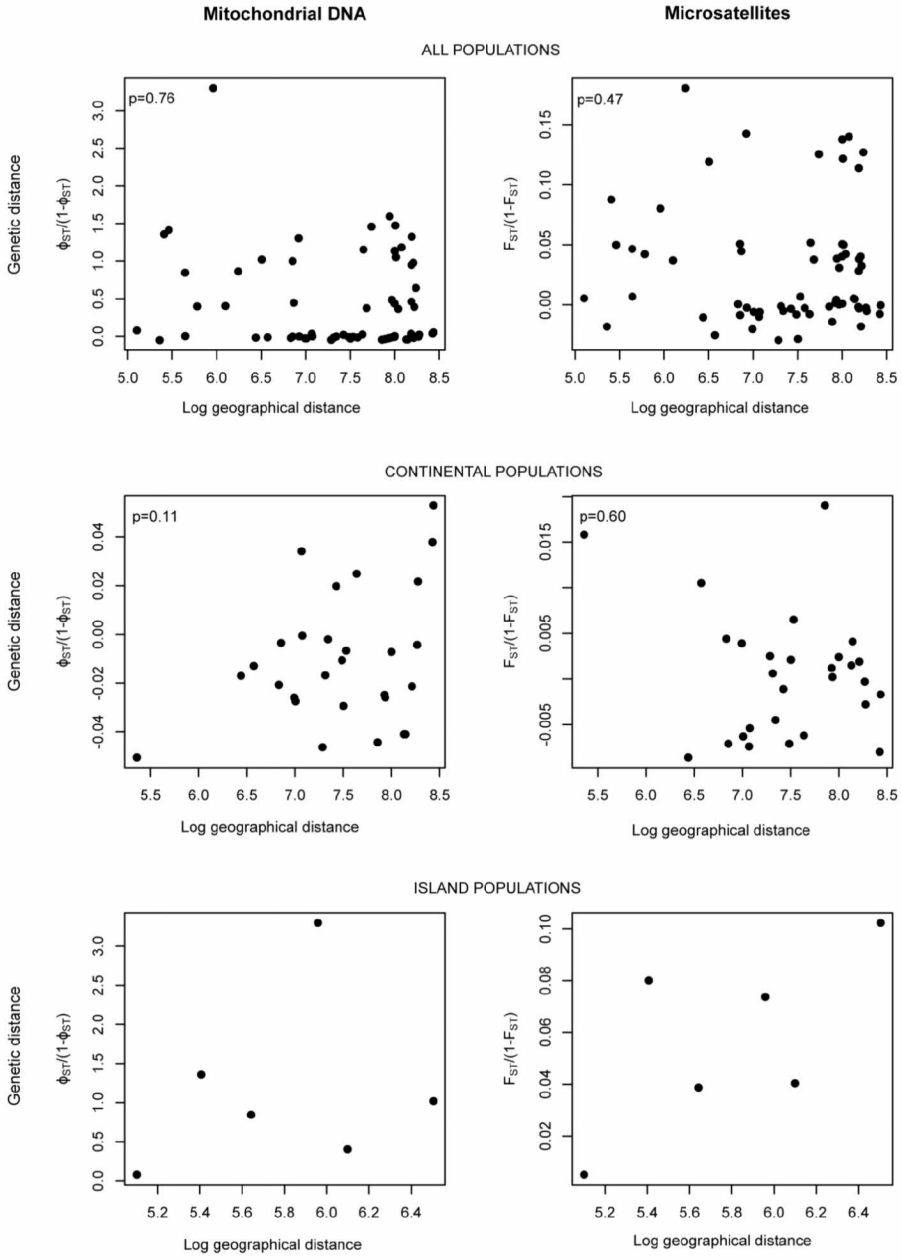

Fig 2. Isolation by distance plots of pairwise population values for log geographic distance and genetic distance

Genetic distance is given by Slatkin's linearised ϕ_{ST} (ϕ_{ST}/(1- ϕ_{ST}) for cytochrome *b* mtDNA analyses (left column) or Slatkin's linearised F_{ST} (F_{ST}/(1-F_{ST}) for microsatellite analyses (right column). Note that the scales vary. Analyses were performed for all *E. helvum* populations (n = 12), for continental populations only (n = 9), or for island populations only (n=4). Statistical significance was assessed using a Mantel test and *p*-values are shown where sample size was sufficient to allow testing. Geographic distance is given in km.

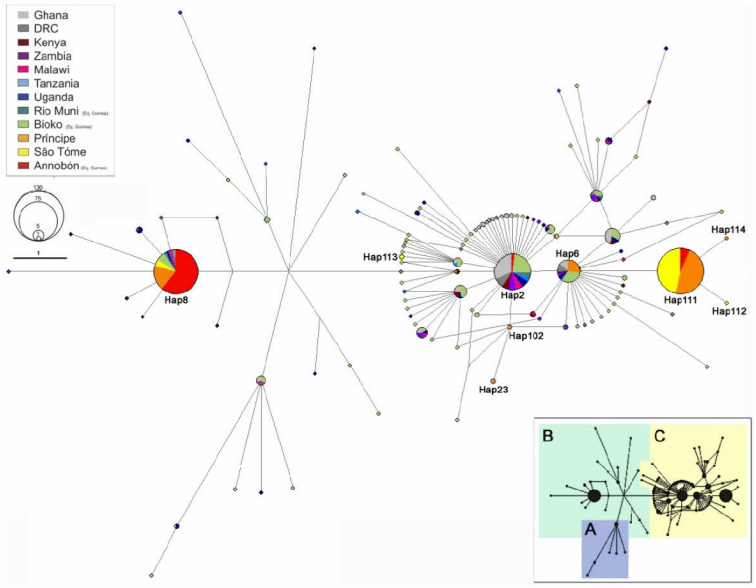

Fig 3. ***Eidolon helvum*** **cytochrome** ***b*** **median joining haplotype network**

No spatial clustering is present in continental African countries or within regions. Each circle represents a unique haplotype, and its size is proportional to its frequency. Lines represent base pair changes between two haplotypes, with the length proportional to the number of base pair changes. Main haplotypes and those containing island samples are labelled by name. Inset in the bottom right shows the relationship between the haplotype network and three clades identified in the Bayesian phylogeny.

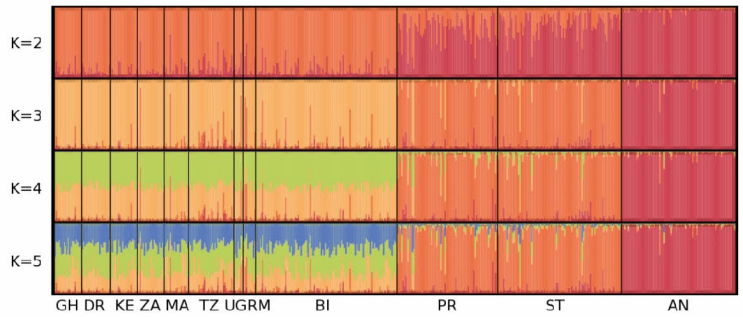

Fig 4. Estimated population structure

Estimates from STRUCTURE analyses for K = 2 to 5 based on microsatellite data from 502 individuals. Analyses run using the admixture setting identified three clusters corresponding to continental and Bioko populations (left), São Tomé and Príncipe (centre, orange) and Annobón (right, red). Each vertical line represents the proportional membership assignment of one individual to each of K coloured clusters. Black lines divide the plot into sampling locations. Ghana (GH), DRC (DR), Kenya (KE), Zambia (ZA), Malawi (MA), Tanzania (TZ), Uganda (UG), Rio Muni (RM), Bioko (BI), Príncipe (PR), São Tomé (ST), Annobón (AN).

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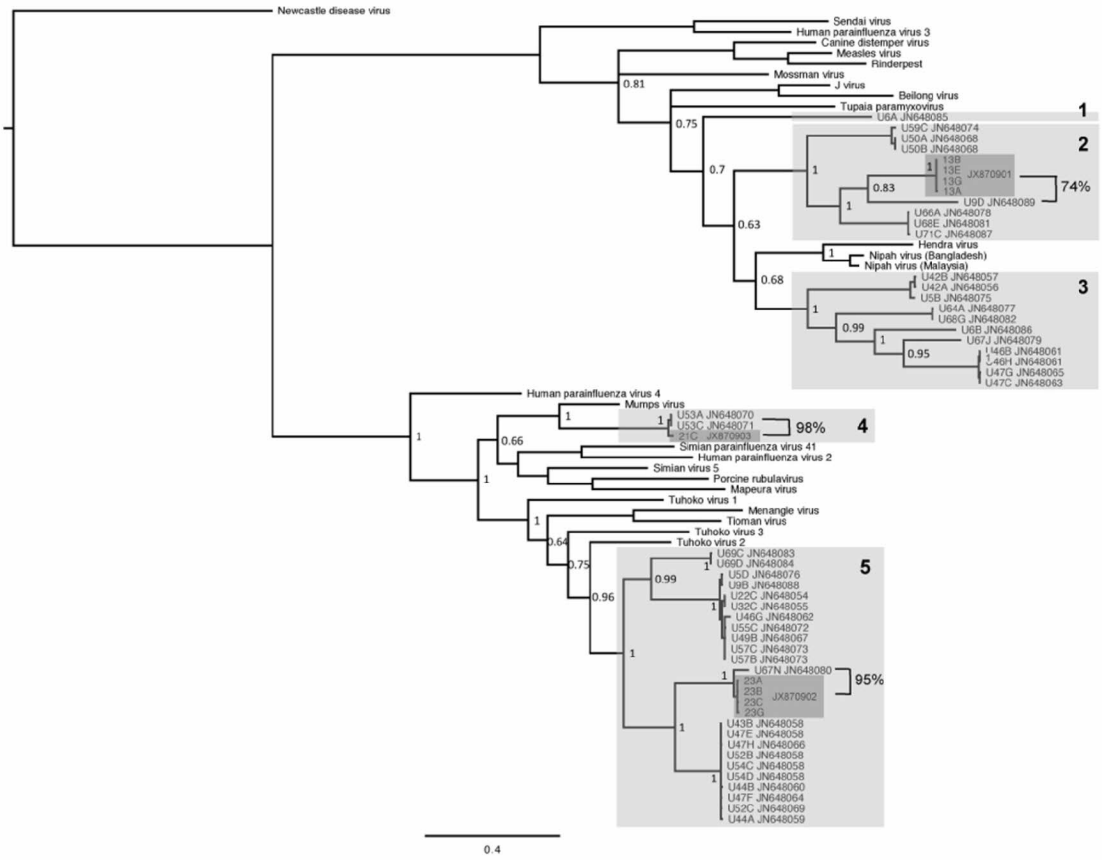

Fig 5. Diversity of paramyxoviruses in *Eidolon helvum* urine collected across multiple African sites detected using *Paramyxovirinae*-targeted PCR

Phylogenetic tree for a 531 bp segment of the polymerase gene of members of the subfamily *Paramyxovirinae*, including sequences generated in this study and publicly available paramyxovirus sequences (with GenBank accession numbers). Relevant posterior probability values are shown. Horizontal branches are drawn to a scale of nucleotide substitutions per site. Individual extraction pools IDs are followed by letters denoting the clone. Groups containing sequences previously uncharacterized sequences that display a common phylogenetic origin supported by high posterior probability values (≥0.95) are highlighted by numbered light grey boxes. Within these boxes, sequences obtained from samples collected from Tanzania and Uganda are further highlighted by darker grey boxes. Pair wise nucleotide identities of the sequences from samples collected Tanzania and Uganda with their nearest phylogenetic relative are shown within the grey boxes. One PCR-positive Ugandan pooled sample (sample 23) contained paramyxoviral sequence with 95% nucleotide sequence identity with sequences detected in Ghana that comprised part of a phylogenetically-distinct lineage of unclassified bat-derived viruses (group 5). Of the two PCR-positive Tanzanian samples, one contained paramyxoviral sequence related to mumps virus (sample 21) and shared 98% nucleotide identity with a Ghanaian sequence (group 2), and the other (sample 13) contained a sequence related to, but distinct from (74% nucleotide identity) sequences detected in Ghana (group 3).

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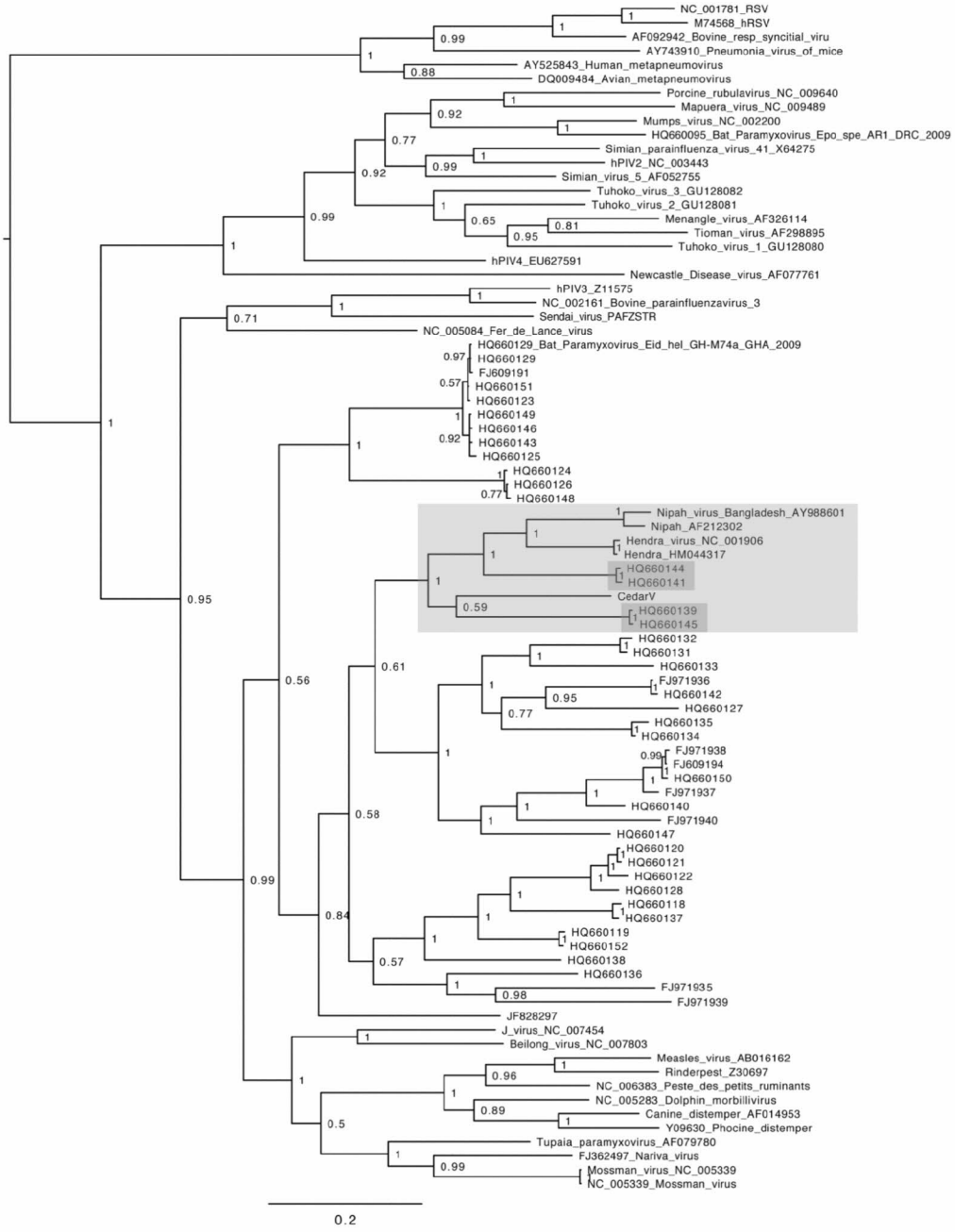

Fig 6. Henipavirus phylogenetic relationships

Phylogeny based on a 559 bp segment of the polymerase gene incorporating fragments known Paramyxovirinae and fragments from Drexler et al[13]. The clade containing known henipaviruses (Hendra virus (HeV), Nipah Virus (NiV) and Cedar virus (CedPV)) is highlighted in pale gray. Sequence fragments from viruses detected in *E. helvum* within this clade are further highlighted by dark gray boxes. Posterior probability values are shown and the bar represents 0.2 expected nucleotide substitutions per site. GenBank accession numbers are shown.

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Table 1

***E. helvum* sample sizes and results for genetics and serological assays for individuals sampled from 12 populations.**

For urine PCRs, results are given as: positive/total tested (* indicates samples collected from single individuals and tested individually, † indicates pooled samples). For serological assays, results are given as: positive/total tested (seroprevalence, 95% confidence interval). Nipah virus (NiV) microsphere binding assay results shown are based on a positive cutoff of MFI>500. Henipavirus virus neutralisation tests (VNTs) were considered positive for neutralisation at dilutions of ≥1:10, and LBV mFAVNs at >1:9. ‡ indicates biased sample sets, where only samples with microsphere binding assay MFI>750 were tested using VNTs.

Country		Sampled	Microsat.	Cyt *b*	Urine PCR	LBV mFAVN	NiV Binding	HeV/NiV VNT
Ghana	**(GH)**	1073	20	64	(ref 15)	**236/745** (31.7%, 28.4-35.1)	**369/954** (38.7%, 35.6-41.8)	**9/61** (14.8%, 8-25.7)
DRC	**(DR)**	34	21	21				
Kenya	**(KE)**	93	20	20				
Zambia	**(ZA)**	125	20	21	0/5*	**6/10** (60%, 31.3-83.2)	**5/12** (41.7%, 19.3-68)	
Malawi	**(MA)**	22	18	18	0/6*	**4/12** (33.3%, 13.8-60.9)	**4/16** (25%, 10.2-49.5)	
Tanzania	**(TZ)**	263	33	34	2/10†	**101/230** (43.9%, 37.7-50.4)	**117/245** (47.8%, 41.6-54.0)	**11/222** (5%, 2.8-8.7)
Uganda	**(UG)**	7	7	7	1/1†	**4/5** (80%, 37.6-99)	**6/7** (85.7%, 48.7-99.3)	
Rio Muni	**(RM)**	10	9	10				
Bioko	**(BI)**	112	104	102		**28/105** (26.7%, 19.1-35.8)	**54/105** (51.4%, 42-60.8)	**16/49‡** (32.7%, 21.2-46.6)
Príncipe	**(PR)**	89	76	70		**23/57** (40.4%, 28.6-53.3)	**27/62** (43.5%, 31.9-55.9)	**11/21‡** (52.4%, 32.4-71.7)
São Tomé	**(ST)**	121	91	94		**42/96** (43.8%, 34.3-53.7)	**48/98** (49%, 39.3-58.7)	**20/39‡** (51.3%, 36.2-66.1)
Annobón	**(AN)**	135	84	83	0/1*	**7/121** (5.8%, 2.8-11.5)	**45/122** (36.9%, 28.8-45.7)	**2/122** (1.6%, 0.5-5.8)
Total		**2013**	**502**	**544**		**451/1381** (32.7%, 30.2-35.2)	**675/1621** (41.6%, 39.3-44.1)	**69/514** (13.4%, 10.7-16.6)

Table 2

Molecular diversity of continental and island *E. helvum* populations.

Diversity statistics were inferred from 397 bp of cytochrome *b* mitochondrial DNA and 16 microsatellites (Population ID (Pop), Number of sequences (*n*), Number of Haplotypes (*nh*), Singleton[1] haplotypes (%), Private[1] haplotypes (%), Haplotype diversity ($h \pm$ Standard deviation), Haplotype richness (HR), Nucleotide diversity ($\pi \pm$ Standard deviation), Molecular diversity (θ_S), Expansion coefficient (S/d), Mean number of alleles per locus (A), Allelic richness (R_S), Private alleles[2] (%), Observed heterozygosity ($H_O \pm$ Standard deviation).

				cytochrome *b* (mtDNA) diversity								Nuclear Diversity			
		Pop	***n***	***nh***	**Singleton (%)**	**Private (%)**	***h* ± SD:**	**HR**	**$\pi \pm$ SD**	**θ_S**	**s/d**	**A**	**R_S**	**Private (%)**	**$H_O \pm$ SD**
Population-level	Continental	**GH**	64	29	51.70%	58.60%	0.89 ± 0.04	4.47	0.007 ± 0.0008	7.4	12.52	9.56	3.95	0.70%	0.75 ± 0.26
		DR	21	11	45.50%	45.50%	0.87 ± 0.06	4.04	0.006 + 0.0011	3.89	5.81	9.19	3.85	0.70%	0.75 ± 0.26
		KE	20	14	57.10%	57.10%	0.94 ± 0.04	4.95	0.009 ± 0.0015	5.92	5.98	9.19	3.93	0.00%	0.76 ± 0.26
		ZM	21	15	53.30%	53.30%	0.94 ± 0.04	5.04	0.010 ± 0.0017	6.11	5.56	8.81	3.89	0.00%	0.75 ± 0.26
		MA	18	11	9.10%	9.10%	0.92 ± 0.05	4.59	0.009 ± 0.0011	4.07	4.1	7.94	3.87	0.80%	0.75 ± 0.25
		TZ	34	23	43.50%	43.50%	0.96 ± 0.02	5.29	0.011 ± 0.0011	7.58	7.21	9.56	3.89	2.60%	0.75 ± 0.25
		UG	7	5	40.00%	40.00%	0.86 ± 0.14	4	0.006 ± 0.0018	3.27	3.23	5.56	4.01	0.00%	0.64 ± 0.39
		RM	10	6	33.30%	33.30%	0.84 ± 0.10	3.73	0.007 ± 0.0014	2.83	2.77	5.81	3.78	2.20%	0.67 ± 0.33
		BI	102	50	66.00%	70.00%	0.95 ± 0.01	5.07	0.008 ± 0.0005	9.24	15.83	12.44	3.87	4.50%	0.74 ± 0.26
	Island	**PR**	70	4	25.00%	50.00%	0.24 ± 0.07	0.77	0.004 ± 0.0010	1.95	7.18	9.69	3.47	0.60%	0.68 ± 0.27
		ST	94	6	16.70%	16.70%	0.53 ± 0.05	1.58	0.007 ± 0.000/	2.08	3.61	9.81	3.45	0.00%	0.68 ± 0.27
		AN	83	3	0.00%	0.00%	0.20 ± 0.06	0.61	0.003 ± 0.0009	1.4	5.34	6.25	2.79	1.00%	0.55 ± 0.31
Regional-level		**ALL**	544	114	75.40%	79.80%	0.87 ± 0.01	NA	0.010 ± 0.0002	13.38	23.19	NA	NA	NA	0.72 ± 0.27
	Cont.	**CT**	195	74	68.90%	79.70%	0.91 ± 0.02	NA	0.008 ± 0.0005	12.31	21.38	NA	NA	10.00%	0.75 ± 0.26
		CB	297	110	76.40%	95.50%	0.92 ± 0.01	NA	0.008 ± 0.0004	14.04	26.3	NA	NA	25.10%	0.75 ± 0.26
	Is.	**ilS**	247	9	22.20%	44.40%	0.56 ± 0.02	NA	0.009 ± 0.0002	2.14	3.71	NA	NA	2.20%	0.66 ± 0.28

[1]Proportion of haplotypes present in a population or region that are singleton (only found in a single individual across all populations) or private (occurring in one or more individual but a single population or region).

[2]Proportion of alleles present in a population or region that occur in a single population or region.

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Table 3
Structure of analyses and results of Analysis of Molecular Variance

Mitochondrial DNA - Cytochrome *b*				
Structure tested	**% Variance**	**ϕStatistics**	**ϕ Statistics**	***p*-value**
1. One Group (All populations)				
Among populations	34.73	$\phi_{ST} = 0.347$	$\phi'_{ST} = 0.358$	**0.00**
Within populations	65.27			
2. One Group (Continental only)				
Among populations	0.62	$\phi_{ST} = 0.006$	$\phi'_{ST} = 0.003$	0.20
Within populations	99.38			
3. Two Groups (Continental vs. Bioko)				
Among groups	−0.32	$\phi_{CT} = -0.003$	$\phi'_{CT} = 0.001$	0.56
Among pops within groups	0.69	$\phi_{SC} = 0.007$	$\phi'_{SC} = 0.004$	0.21
Among pops among groups	99.63	$\phi_{ST} = 0.004$		0.16
4. One Group (Príncipe, São Tomé and Annobón islands)				
Among populations	56.25	$\phi_{ST} = 0.562$	$\phi'_{ST} = 0.575$	**0.00**
Within populations	43.75			
5. Two Groups (Continental + Bioko) vs. (Príncipe, São Tomé and Annobón islands)				
Among groups	15.80	$\phi_{CT} = 0.158$	$\phi'_{CT} = 0.162$	0.13
Among pops within groups	23.42	$\phi_{SC} = 0.278$	$\phi'_{SC} = 0.288$	**0.00**
Among pops among groups	60.78	$\phi_{ST} = 0.392$		**0.00**
6. Two Groups (Príncipe and São Tomé) vs. Annobón				
Among groups	61.85	$\phi_{CT} = 0.619$	$\phi'_{CT} = 0.633$	0.33
Among pops within groups	3.22	$\phi_{SC} = 0.084$	$\phi'_{SC} = 0.086$	**0.01**
Among pops among groups	34.93	$\phi_{ST} = 0.651$		**0.00**
7. Three Groups (Continental + Bioko) vs. (Príncipe + São Tomé) vs. (Annobón)				
Among groups	42.46	$\phi_{CT} = 0.425$	$\phi'_{CT} = 0.436$	**0.00**
Among pops within groups	1.46	$\phi_{SC} = 0.025$	$\phi'_{SC} = 0.025$	**0.00**
Among pops among groups	56.08	$\phi_{ST} = 0.439$		**0.00**
8. Four Groups (Continental + Bioko) vs. (Príncipe) vs. (São Tomé) vs. (Annobón)				
Among groups	41.63	$\phi_{CT} = 0.416$	$\phi'_{CT} = 0.427$	**0.00**
Among pops within groups	0.77	$\phi_{SC} = 0.013$	$\phi'_{SC} = 0.012$	0.16
Among pops among groups	57.60	$\phi_{ST} = 0.424$		**0.00**

Microsatellites

Structure tested	% Variance	*F*-Statistics	*F*'-Statistics	*p*-value
1. One Group (All populations)				
Among populations	4.28	F_{ST} = 0.043	F'_{ST} = 0.207	**0.00**
Within populations	95.72			
2. One Group (Continental only)				
Among populations	-0.22	F_{ST} = -0.002	F'_{ST} = 0.007	0.96
Within populations	100.22			
3. Two Groups (Continental vs. Bioko)				
Among groups	0.60	F_{CT} = 0.006	F'_{CT} = 0.085	0.22
Among pops within groups	-0.90	F_{SC} = -0.009	F'_{SC} = 0.002	1.00
Among pops among groups	100.30	F_{ST} = -0.003		1.00
4. One Group (Príncipe, São Tomé and Annobón islands)				
Among populations	4.45	F_{ST} = 0.045	F'_{ST} = 0.133	**0.00**
Within populations	95.55			
5. Two Groups (Continental + Bioko) vs. (Príncipe, São Tomé and Annobón islands)				
Among groups	4.01	F_{CT} = 0.040	F'_{CT} = 0.187	0.01
Among pops within groups	1.88	F_{SC} = 0.020	F'_{SC} = 0.118	**0.00**
Among pops among groups	94.11	F_{ST} = 0.059		**0.00**
6. Two Groups (Príncipe and São Tomé) vs. Annobón				
Among groups	5.44	F_{CT} = 0.054	F'_{CT} = 0.140	0.33
Among pops within groups	0.72	F_{SC} = 0.008	F'_{SC} = 0.033	**0.00**
Among pops among groups	93.83	F_{ST} = 0.062		**0.00**
7. Three Groups (Continental + Bioko) vs. (Príncipe, São Tomé) vs. (Annobón)				
Among groups	6.04	F_{CT} = 0.060	F'_{CT} = 0.192	**0.00**
Among pops within groups	-0.08	F_{SC} = 0.000	F'_{SC} = 0.063	0.97
Among pops among groups	94.04	F_{ST} = 0.060		**0.00**
8. Four Groups (Continental + Bioko) vs. (Príncipe) vs. (São Tomé) vs. (Annobón)				
Among groups	5.90	F_{CT} = 0.059	F'_{CT} = 0.151	**0.01**
Among pops within groups	-0.34	F_{SC} = -0.004	F'_{SC} = 0.094	1.00
Among pops among groups	94.44	F_{ST} = 0.056		**0.00**

Transboundary and Emerging Diseases

REVIEW ARTICLE

Assessing the Potential Role of Pigs in the Epidemiology of Ebola Virus in Uganda

C. Atherstone[1], E. Smith[1], P. Ochungo[2], K. Roesel[1] and D. Grace[2]

[1] International Livestock Research Institute, Kampala, Uganda
[2] International Livestock Research Institute, Nairobi, Kenya

Keywords:
Ebola; pigs; Uganda; epidemiology; risk assessment

Correspondence:
C. Atherstone. International Livestock Research Institute, Plot 106 Katalima Rd, Naguru. P.O Box 24384, Kampala, Uganda.
Tel.: +256 392 081154/5; Fax: +254 20 422 3001;
E-mail: c.atherstone@cgiar.org

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Summary

Uganda has experienced 4 Ebola outbreaks since the discovery of the virus. Recent epidemiological work has shown pigs are hosts for Ebola viruses. Due to their high reproduction rates, rapid weight gain, potential to provide quick financial returns and rising demand for pork, pig production in Uganda has undergone massive expansion. The combination of pork sector growth supported by development programmes and Ebola virus risk prompted a foresight exercise using desk, interview and spatial methods. The study found that the lack of serological evidence for specific reservoir species, the number of human index cases unable to account for their source of infection, domestic pig habitat overlap with potential Ebola virus zoonotic host environments, reported interactions at the human–pig–wildlife interface that could support transmission, fever in pigs as a commonly reported problem by pig farmers and temporal correlation of outbreaks with peak pork consumption periods warrants further research into potential zoonotic transmission in Uganda from pigs.

Introduction

During the last decades, the demand for meat and milk has increased, particularly in developing countries where consumption of meat increased almost three times more than in developed countries (Delgado, 2003). Pig production is becoming increasingly popular, with pork and poultry contributing 76% of the increased meat consumption in the developing world between 1982 and 1998 (Delgado et al., 2001).

In sub-Saharan Africa, millions of small scale farmers efficiently supply the great majority of the meat, milk and fish markets. Animal source food products have a high nutritional value which enhances public health, while the production, transportation, processing and retailing of these products provide income and employment to millions.

Over the past three decades, the reported pig population has increased 1500%, from 0.19 to 3.2 million in Uganda (Uganda Bureau of Statistics, 2008). In 2011, Uganda had the highest per capita consumption of pork in East Africa at 3.4 kg/person/year (Ouma et al., 2014). More than 1.1 million poor households in Uganda own pigs, mostly managed by women and children in backyard activities. Indeed, 80% of pig production in Uganda is carried out by smallholder crop-livestock farmers (Ouma et al., 2014). Despite this dependence on livestock, there is a strong association between poverty, hunger, livestock keeping and zoonoses (Grace et al., 2012).

Furthermore, pigs are the only domestic livestock species presently known to be naturally infected with Ebola viruses (Barrette et al., 2009). The disease course in pigs depends on infective strain; Reston Ebola virus (REBOV) causes asymptomatic signs to mild respiratory symptoms (Barrette et al., 2009), and Zaire Ebola virus (ZEBOV) causes fever and severe lung pathology (Kobinger et al., 2011).

In Uganda, the International Livestock Research Institute (ILRI) aims to support development of the pig value chains through risk-based approaches to ensure food safety. A risk assessment and risk map to determine the threat Ebola viruses, poses in the pig value chains in Uganda was warranted.

Materials and Methods

Relevant articles in the published and grey literature were identified using online databases, visiting university libraries and interviewing experts within Uganda. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used for the retrieval and screening of all articles (Moher et al., 2009). Databases searched included PubMED, CabDirect, Web of Science, African Journals Online, Makerere University library, World Bank, World Health Organization (WHO) Global burden of disease, World Animal Health Information Database (WAHIS), Health map, International Symposia on Veterinary Epidemiology and Economics proceedings, Tropentag proceedings, ILRI repository, International Food Policy Research Institute resources, WHO library database (WHOLIS), International System for Agricultural Science and Technology (AGRIS), Centres for Disease Control & Prevention (CDC) and two national daily newspapers (Daily Monitor and New Vision). Relevant searches using the search terms 'Uganda' AND 'Ebola', 'Ebola' AND 'pig' OR 'pork' OR 'porcine' OR 'swine' were performed on all the above databases and websites.

Pig distribution in Uganda (see Fig. 1) is part of the global distribution of livestock mapping that was carried out by the Food and Agriculture Organization (FAO) (Robinson and Wint, 2014).

An additional fifteen expert interviews were conducted using a semistructured questionnaire. Finally, unpublished student theses (Bachelor, Masters and PhD) at Makerere University, College of Veterinary Medicine, Animal Resources and Biosecurity, were reviewed from 1990 to the present for relevant content on Ebola virus in pigs or diseases in pigs that shared clinical symptoms or histopathological changes similar to Ebola virus infection in pigs.

To investigate the interactions of domestic pigs with wildlife, a questionnaire was administered to seventeen animal health professionals with regular field experience in ten geopolitical regions that also reflect basic agro-ecological zones in Uganda.

A risk map investigating the spatial overlap of known factors supporting potential zoonotic Ebola virus spillover from animals to humans in Uganda was developed using ArcGIS version 10.2. Risk was represented binomially as high or low in terms of (i) suitable zoonotic niche for Ebola viruses in Uganda at 5 × 5 km pixel unit (Pigott et al., 2014), (ii) number of pigs per square kilometre at 1 × 1 km pixel unit (Robinson et al., 2007; Robinson and Wint, 2014) and (iii) number of people living in extreme poverty (1.25 USD/day) at 10 × 10 km pixel unit (Wood et al., 2010). All data layers were resampled to 1 × 1 km pixel unit before overlaying. The threshold for high pig density and human poverty distribution in Uganda was described as above the median which was 0 pigs/km^2 and 833 people living in extreme poverty/10 km^2, respectively. As such, 'high' pig density is essentially a reflection of the presence and absence of pig keeping in the country.

Poverty has been considered a factor for increased risk for zoonotic disease transmission of Ebola virus and other diseases (Grace et al., 2012; Bausch and Schwarz, 2014). Therefore, the use of poverty as a risk was justified due to recent evidence suggesting its link to bush meat trade (Wolfe and Daszak, 2005) and increased animal contact (Paige et al., 2014). In addition, protected forested areas were represented on the map due to the occurrence of higher human population at the edges of protected areas (Pourrut et al., 2005; Becquart et al., 2010) and the relevance of forested areas for potential zoonotic transmission (Monath, 1999; Peterson et al., 2004; Pourrut et al., 2005; Leroy et al., 2007; Becquart et al., 2010; Switzer and Tang, 2012).

The zoonotic niche data were adapted from a recent mapped model of the predicted environmental suitability for zoonotic transmission of Ebola virus in Africa (Pigott et al., 2014). We converted the continuous probability of risk to a binary map classifying pixels as either high or low risk.

Results and Discussion

The systematic literature review identified the primary factors that support potential zoonotic transmission of Ebola virus in Uganda from pigs as (i) the lack of serological evidence for presumed reservoir species, particularly in Uganda, (ii) the number of human index cases unable to account for their source of infection, particularly in Uganda, (iii) domestic pig habitat overlap with potential Ebola virus zoonotic host environments, (iv) reported interactions at the human–pig–wildlife interface that could support transmission, (v) fever in pigs as a commonly reported problem by pig farmers and 6) temporal correlation of outbreaks with peak pork consumption periods.

The lack of serological evidence for specific reservoir species, particularly in Uganda

Anecdotal evidence suggests bats as a potential reservoir host of Ebola virus. In the 1976 outbreak of Sudan Ebola virus (SUDV), the first six human cases were cotton factory employees who worked in a room where bats roosted (Leroy et al., 2009). In 1994, in Côte d'Ivoire, chimpanzees which developed Ebola virus disease (EVD) had been feeding in a fig tree together with fruit bats for 2 weeks before developing the disease (Formenty et al., 1999). The 1989–1990 and 1996 REBOV outbreaks in primate facilities were linked back to a single export facility in the Philippines

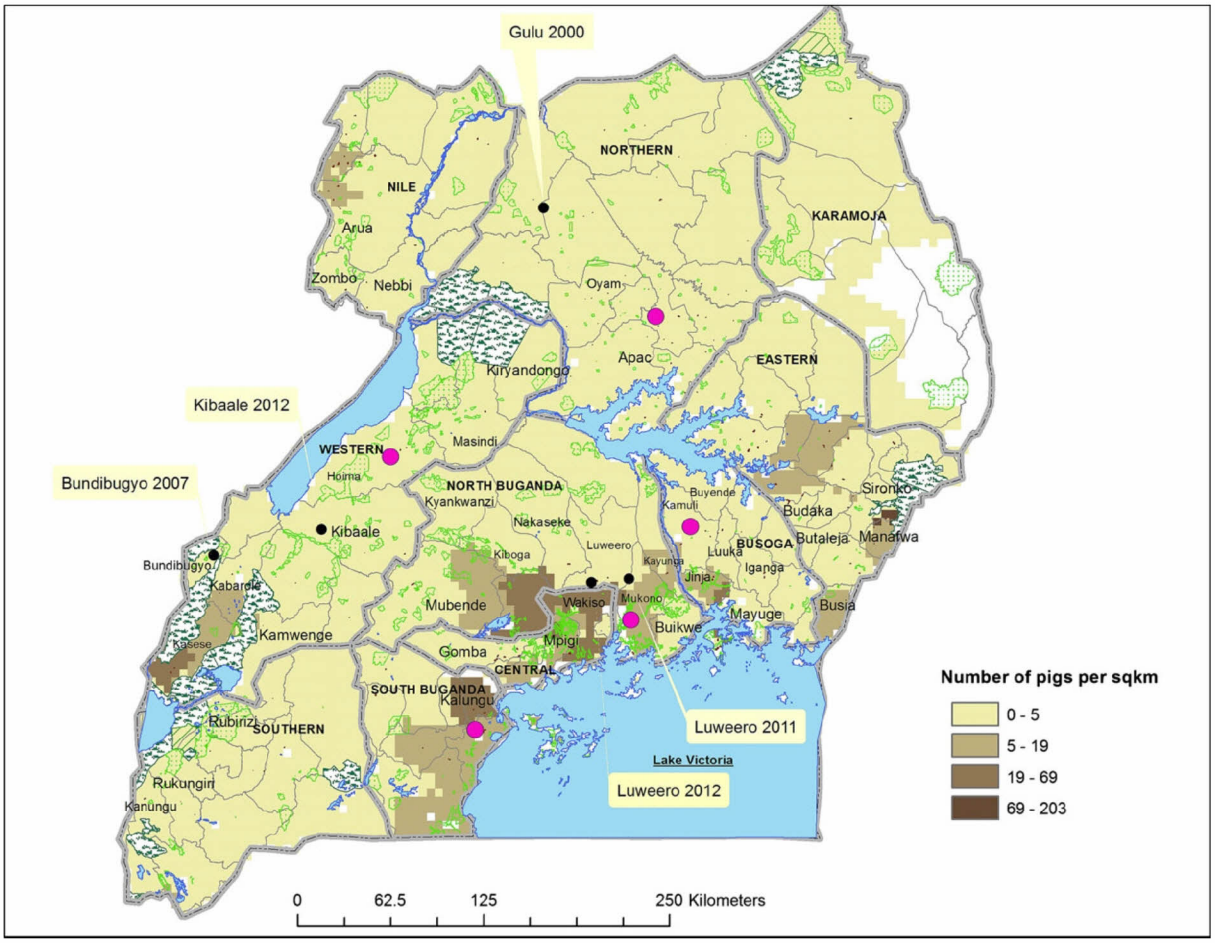

Fig. 1. Pig distribution in Uganda.

which was a former fruit orchard where animals were potentially exposed to fruit bats (Goldsmith, 2010). In addition, the index case in the 2007 ZEBOV outbreak in DRC was linked to direct exposure to freshly killed bats bought from hunters (Leroy et al., 2009).

The serological evidence for fruit bats as the reservoir species for Ebola virus is compelling (Reed, 2012; Olival and Hayman, 2014); however, there are still distinct gaps in knowledge. To date, there have been no successful attempts at Ebola virus isolation in any bat species (see Table 1). Three main species have consistently been found antibody and PCR positive to ZEBOV in Africa and are thus presumed to be natural reservoirs of Ebola virus: Franquet's epauletted fruit bat (*Epomops franqueti*), little collared fruit bat (*Myonycteris torquata*) and the hammer-headed fruit bat (*Hypsignathus monstrosus*) (Leroy et al., 2005; Olival and Hayman, 2014). Pilot work in Uganda has found *Epomophorus labiatus*, *Rousettus aegyptiacus* and *Eidolon helvum* ZEBOV seropositive (Reed, 2012). However, there are currently no confirmed bat hosts for the Sudan and Bundibugyo Ebola viruses found in Uganda.

Despite speculations on other potential mammalian reservoir species (Peterson et al., 2004) and large post-outbreak ecological sampling of wildlife and domestic species in Africa (Olson et al., 2012), there is limited and inconsistent serological evidence for other species involvement. The main wildlife species found to have evidence of harbouring Ebola viruses includes non-human primates, duikers, dogs and small rodents and shrews (Olson et al., 2012).

Presently, pigs are the only livestock species found naturally infected with REBOV (Barrette et al., 2009) and have been experimentally infected with ZEBOV (Kobinger et al., 2011). Surveillance in endemic Africa has been limited, with only two sampling efforts reported with very small sample sizes – 12 samples from two outbreaks in DRC 1976 and 1995 (Olson et al., 2012) and 31 samples from the 2012 Kibaale outbreak in Uganda (personal communication, Dr. Trevor Shoemaker). No serological evidence for

Table 1. Bat species found Ebola virus positive by serology or PCR [adapted from (Bausch and Schwarz, 2014)]

Virus	Bat species	Detection method
Reston Ebola virus	*Cynopterus sphinx*Greater short-nosed fruit bat	Antibodies
	*Hipposideros pomona*Pomona roundleaf bat	Antibodies
	*Miniopterus schreibersii*Common bent wing bat	Antibodies
	*Myotis pilosus*Rickett's big-footed bat	Antibodies
	*Pipistrellus pipistrellus*Common pipistrelle	Antibodies
	*Rousettus amplexicaudatus*Geoffrey's rousette	Antibodies
	*Rousettus leschenaultia*Leschenault's rousette	Antibodies
Zaire Ebola virus	*Eidolon helvum*Straw-coloured fruit bat	Antibodies
	*Epomps franqueti*Franquet's epauletted fruit bat	Antibodies, PCR
	*Epomphorus gambianus*Gambian epauletted fruit bat*	Antibodies
	*Hypsignathus monstrosus*Hammer-headed bat	Antibodies, PCR
	*Micropteropus pusillus*Peters's dwarf epauletted fruit bat	Antibodies
	*Tadarida condylura*Angolan free-tailed bat	Antibodies
	*Myonycteris torquata*Little collared fruit bat	Antibodies, PCR
	*Rousettus aegyptiacus*Egyptian fruit bat	Antibodies
	*Rousettus leschenaultii*Leschenault's rousette*	Antibodies
	*Epomophorus wahlbergi*Wahlberg's epauletted fruit bat	Experimental infection

*Not found in Uganda.

the endemic African species of Ebola virus in pigs currently exists. Anecdotal accounts of widespread pig deaths before outbreaks have been reported, (Katatyi, 2014), but analysis into cause of death has not been reported.

The number of human index cases unable to account for their source of infection, particularly in Uganda

There have been a number of human index cases of EVD in previous outbreaks with no known contact with non-human primates or bats (Table 2) fuelling investigations for other zoonotic reservoirs. In the 1976 outbreak in Sudan 4.9% (14/284) of cases and 17.4% (55/216) of cases during the 1996 outbreak in Kikwit, DRC had no direct physical contact with an infected person or known infected carcass (Roels and Bloom, 1999; Allela et al., 2005). The source of infection in the 2007 Bundibugyo outbreak (Butagira et al., 2007) and 2012 Kibaale outbreak (ProMED-mail 2012) was speculated as contact with a monkey, but remains unconfirmed in these outbreaks and unknown in all other outbreaks in Uganda.

Domestic pig habitat overlap with potential Ebola virus zoonotic host environments

In Uganda, mixed cropping-livestock subsistence systems are often interspersed with forested or woodland mosaic landscapes which are suitable fruit bat and non-human primate habitats (Herrero et al., 2009). These landscapes are particularly prevalent in the central and western part of the country which roughly correlates with pig distribution in Uganda (Fig. 1). Of the eight fruit bat species found in Uganda and suspected of being Ebola virus reservoirs, seven of them are found in forest or rainforest biomes with four of them having documented associations with agricultural or urban/suburban habitats (IUCN 2013). A study from the western region in Uganda found that subsistence farmers and those living near small patches of remnant forests (0.5–3 km^2) had increased incidences of general contact including pigs and primates (Paige et al., 2014). This anthrobiome is a suitable setting for the hypothesized zoonotic Ebola virus epidemiological cycle involving pigs. Pig production occurs in all previous Ebola virus outbreak districts at a density of at least 0–5 pigs/km^2. Furthermore, four of the five outbreak districts occur in regions where there are areas of >19 pigs/km^2 (Fig. 2).

Reported interactions at the human–pig–wildlife interface that could support transmission

Suggested modes of transmission between non-human primates, fruit bats and pigs include competition for fruit leading to spatiotemporal clustering of these frugivorous animals creating an increased likelihood of spillover (Bausch and Schwarz, 2014).

This scenario is supported by the questionnaire respondents who reported domestic pigs having shared feeding spots with bats and primates (Fig. 2); mainly in competition around fruiting trees. Banana plantations near houses and gardens were specific locales where this occurred because these species feed on weeds, fallen banana fruit, household refuse and a number of other plants that grow in the banana plantation.

Table 2. Source of infection for confirmed Ebola virus disease (EVD) cases

Virus strain	Date	Location	Human index case source of infection	# of cases	Case fatality rate (%)
Zaire	1976	Zaire	Unknown	318	88
	1977	Zaire	Unknown	1	100
	1994	Gabon	Contact with NHPs	49	65
	1995	DRC	Unknown	315	88
	1996	Gabon	Contact with NHPs	37	57
	1996	Gabon	Contact with NHPs	60	75
	2001	Gabon/ROC	Contact with NHPs	123	79
	2002	ROC	Contact with NHPs	143	90
	2003	ROC	Contact with NHPs	35	83
	2004	Russia	Lab accident	1	100
	2005	ROC	Unknown	12	75
	2007	DRC	Contact with bats	264	71
	2008	DRC	Unknown	32	47
	2013	Guinea, Liberia, Mali, Nigeria, Senegal, Sierra Leone, Spain, UK, USA	Contact with bush meat unspecified	>24 000	Ongoing in Guinea, Liberia, Sierra Leone
	2014	DRC	Contact with bush meat unspecified	66	74
Sudan	1976	Sudan	Unknown	284	53
	1976	England	Lab accident	1	0
	1979	Sudan	Unknown	43	65
	2000	Uganda (Gulu)	Unknown. 12 index cases identified. Media rumours of being brought by insurgent rebels from Sudan/Ugandan soldiers from DRC through trading baboon and monkey meat	425	53
	2004	Sudan	Unknown	17	42
	2011	Uganda (Luwero)	Unknown. Single case 12-year-old girl. Several species of bats were found several classrooms of the village schoolhouse where the girl attended classes and in unoccupied houses near her home.	1	100
	2012	Uganda (Kibaale)	Unknown. First cases in area close to Kibaale National Park	24	71
	2012	Uganda (Luwero)	Unknown. Motorcycle taxi driver	7	57
Côte d'Ivoire	1994	Côte d'Ivoire	Necropsy of chimp	1	0
Bundibugyo	2007	Uganda (Bundibugyo)	Unknown. Suspected index case 26-year-old pregnant woman. Hunting spears found at house but hunting practice denied	102	42
	2012	DRC	Unknown	36	36

Furthermore, aggressive interactions were reported by some between domestic pigs and primates (Fig. 2), presumably involving competition for food. Considering the deficit of knowledge regarding the host species of Ebola virus in the wild, it is interesting to note the different potential transmission routes between domestic pigs and a variety of wildlife which may play a role in the epidemiology of the virus.

Furthermore, domestic pigs are often left to roam free in Uganda (Ouma et al., 2014), so their habit range can extend beyond smallholder farm perimeters where they encroach on various habitats. Collared free-ranging domestic pigs in western Kenya travelled an average of 4340 m in a 12 h period and had a mean home range of 10 343 m^2 (Thomas et al., 2013). In this study, free-ranging domestic pigs travelled large distances, throughout the day and night, with almost half of the time spent outside their homestead, extending the geographic range and habitats these pigs scavenge and travel in. This large range and lengthier scavenging timeframe could be risk factors for Ebola virus

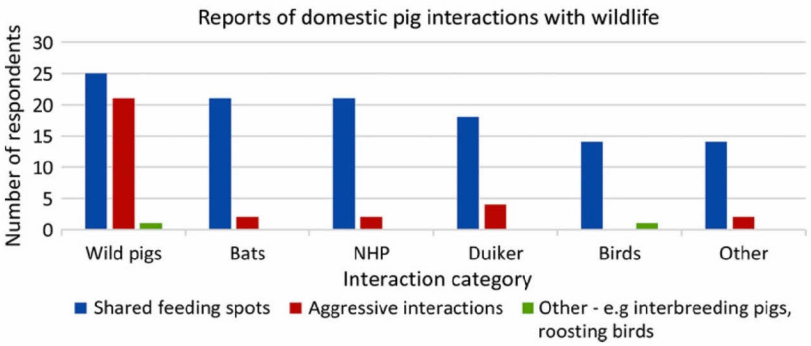

Fig. 2. Reports of domestic pig interactions with wildlife.

infection both between domestic pigs and between domestic pigs and wildlife.

In fact, animal health professionals reported wildlife in livestock areas as being common across all regions of Uganda (Fig. 3). Primates were the most common species group reported to frequent livestock areas, followed by bats and wild pigs. Regions where primates, bats and wild pigs are common and where domestic pigs occur, include western, Nile and northern. Wild pigs were reported as most common in the western region, where two of the past Ebola virus outbreaks have occurred.

Questionnaire respondents reported human interactions with bush pigs and common warthogs across all regions as a very occasional to several times a month or year occurrence (Fig. 3). Physical contact between humans and pigs was reported with attacks by pigs during the breeding season and when people intervened to stop pigs from raiding crops. Coming into contact with sick pigs or their bodily fluids in the environment through shared food resources (such as eating contaminated fruits or tubers uprooted by pigs) particularly in the dry season was also reported. Similar interactions were reported very occasionally for forest hogs and red river hogs from specific regions only. The same pattern is reflected in bush meat practice – bush pigs and common warthogs are eaten by few households in all regions throughout the year or in some regions only when food is scarce. Forest hogs and red river hogs show similar frequency of harvesting but in specific regions only.

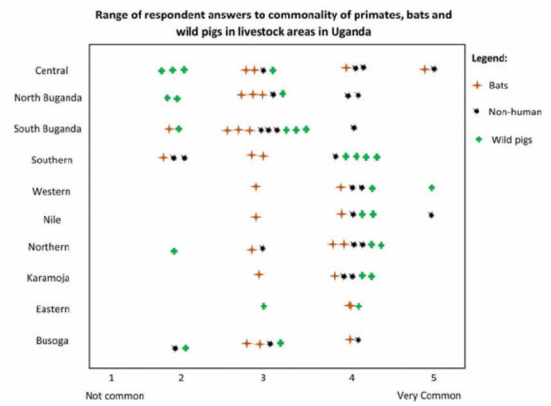

Fig. 3. Range of respondent answers to commonality of primates, bats and wild pigs in livestock areas in Uganda.

Fever in pigs a common problem reported by pig farmers

Pig fever is commonly reported by pig farmers in Uganda. Fever in pigs is attributed to African swine fever (ASF), a lethal haemorrhagic viral disease that produces fever and respiratory signs among others. Some of these clinical signs are similar to pigs experimentally infected with ZEBOV (Kobinger et al., 2011). Considering the inconsistent and under-resourced diagnostic capability for pig pathogens in Uganda and the potential low disease prevalence thought to exist in the suspect reservoir hosts for Ebola virus and other viral haemorrhagic fevers (Reed, 2012; Olival and Hayman, 2014), it is possible that Ebola virus infection in pigs goes undetected and is mistaken for other infections in pigs that cause similar symptoms.

Temporal correlation of outbreaks with peak pork consumption periods

It is typical for meat to be consumed on special occasions in Uganda. Producers and consumers eat pork, especially for Easter and Christmas (Roesel et al., 2014). Overlaying Ebola virus outbreaks in Uganda with seasonal pork consumption patterns shows outbreaks near peak pork consumption periods (see Fig. 4), where increased handling, butchering and transporting of pigs would happen.

ASF epidemiology research in Uganda highlighted how the sale of sick pigs and the sale and consumption of pork from the dead pigs spread and extended an outbreak of ASF in Gulu (Tejler, 2012). This outbreak of ASF happened near Independence Day, a national holiday where meat is typically consumed. In addition, the research doc-

umented that many of the pigs that died as a result of the ASF outbreak were consumed either on the farm or sold to the butcher in the local community. Both the practice of eating pigs that have died of unknown causes and the sale of sick pigs would also spread and extend an outbreak of Ebola virus in pigs and increase the risk of spillover to humans.

Given that pork is a luxury item consumed for special occasions such as public holidays, the potential risk for zoonotic Ebola virus transmission from pigs to humans may be seasonal, linked with periods of greater pork consumption, and hence live pig sales and movement. The highest hypothesized risk is at farm level via direct contact with infected bodily fluids and during slaughtering, where contact with blood, internal organs and other bodily fluids is a part of the processing. At the household consumption level, the hypothesized risk of Ebola virus infection is most likely only if the raw pork is handled in preparation for cooking. Eating of processed pork does not pose a significant risk at present, based on current knowledge of Ebola virus stability and pork cooking and preservation techniques.

The role pigs play in the ecology and epidemiology of Ebola virus is unknown. Several hypothetical possibilities based on other relevant research findings are as follows.

Pig–wildlife–interface

Competition for fruit is a potential interface for transmission of Ebola virus between wildlife and pigs. In fact, questionnaire respondents reported shared feeding spots between pigs, bats and non-human primates.

Shared feeding around fruiting trees is a suitable environment for interspecies transmission from infected saliva deposited on fruit (Pourrut et al., 2009). Given the seasonal variance of antibody prevalence in bat species (Pourrut et al., 2009) and seasonality of fruiting trees, this could result in temporal patterns of transmission between bats and pigs.

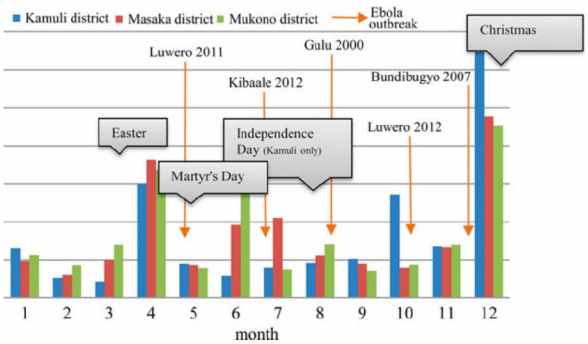

Fig. 4. Seasonality of pork consumption in three districts in Uganda. Note: None of the districts surveyed for consumption trends have reported Ebola virus outbreaks.

Questionnaire respondents reported some aggressive encounters between pigs and non-human primates. Aggressive encounters occur around food resources and non-human primate hunting of pigs. Chimpanzees are known to hunt bushpigs (Goodall, 1986; Boesch and Boesch, 1989; Uehara et al., 1992; Stanford and Wallis, 1994; Stanford, 1996), which is characterized by opportunity or snatch-and-run hunts (Stanford and Wallis, 1994). This contact through hunting could transmit Ebola virus from infected pigs to chimpanzees.

Between pigs

Experimentally infected pigs spread ZEBOV to naïve pigs presumably through aerosols from the oronasal mucosa, which were found to have high titres of ZEBOV (Kobinger et al., 2011). Contact pigs had a less severe disease course than pigs that had been experimentally infected.

The selling of sick and dead pigs, as evidenced during the ASF outbreak in Gulu, are practices that increase the risk of spreading Ebola virus to humans and other pig farms. During the ASF outbreaks, sick pigs and contact pigs were transported 500 km through several districts in Uganda (Tejler, 2012), creating suitable dynamics for secondary outbreaks and extension of the geographic range of Ebola virus outbreaks.

Furthermore, spread between domestic pigs and their wild counterparts, potentially amplifying the virus is possible. Uganda is the natural habitat for several widespread wild pig species: giant forest hog (*Hylochoerus meinerthageni*), Red River hog (*Potamochoerus porcus*), bushpig (*Potamochoerus larvatus*) and common warthog (*Phacochoerus africanus*). Bushpigs in particular have a wide distribution throughout East Africa, where they live and move at the interface of national parks and farmland. This interaction increases pathogen sharing between wild and domestic pigs (Blomström et al., 2012). While transmission dynamics for Ebola virus have not been studied between wild and domestic pigs, possible routes may be through direct contact, contact with urine and faeces in the environment and sharing of food particularly during scavenging. Certainly, the large distances travelled by free-ranging domestic pigs for scavenging could be a risk factor for infection through direct contact, both between domestic pigs and between wild and domestic pigs.

Genotypic evidence of breeding between wild and domestic pigs is being investigated at Makerere University School of Population and Molecular Genetics in Uganda, as phenotypic evidence has been speculated here and elsewhere in Sub-Saharan Africa. This inter-breeding could facilitate Ebola virus transmission.

From pigs to humans

The intensification of pig production combined with poor pig husbandry and slaughter practices rampant throughout Uganda makes pig to human transmission a possibility. Direct contact with infected pigs by farmers, particularly through daily care, butchering and hunting provides contact with bodily fluids and other infectious fluids. Transmission is also possible through contact with contaminated inanimate objects or vegetation (Leroy et al., 2009). A study on animal contacts in the western district of Uganda revealed pigs contributed to 5.6% of reported animal injures to people in the area surrounding Kibaale National Park, this compared to 1.7% by primates (Paige et al., 2014).

In addition to direct contact, aerosol transmission between pigs and humans is a possibility that needs further exploration. Pig farmers in the Philippines were found to be seropositive to REBOV, despite not being involved with slaughtering pigs or having any known contact with infected carcasses (Barrette et al., 2009). In an experimental study, pigs efficiently transmitted ZEBOV via aerosol to primates in conditions resembling a farm setting (Weingartl et al., 2012). These results support transmission of Ebola virus between pigs and primates and provide evidence that transmission from pigs to humans needs to be considered, considering the similar pathogenesis of EVD in primates and humans.

Future directions

To support additional research, a risk map was created to identify the potential high-risk areas suitable for targeted porcine sampling. The potential areas of high risk as defined by zoonotic Ebola virus niche, pig production and high numbers of people living in poverty are shown in Fig. 5. It is important to note that high-risk areas indicate

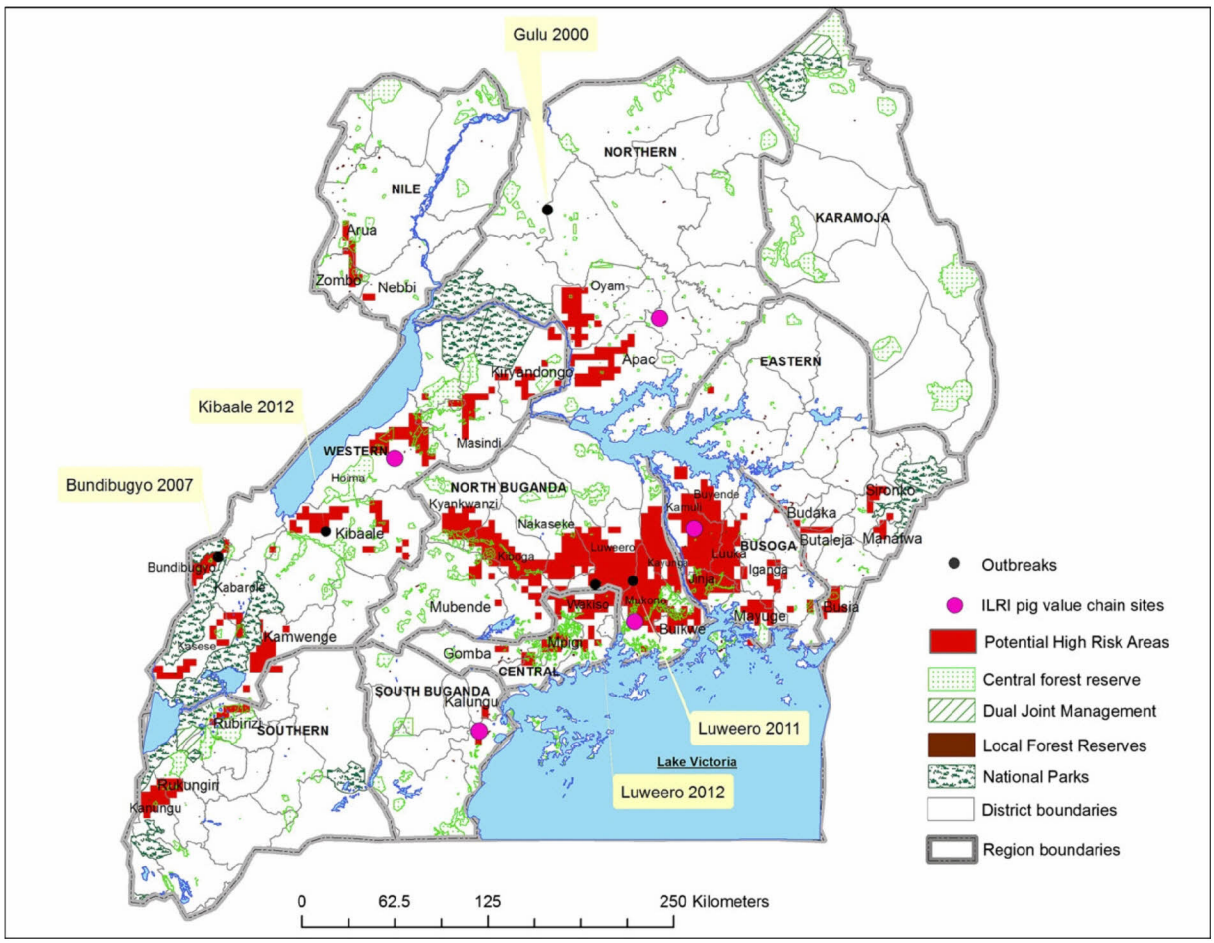

Fig. 5. High-risk areas according to the spatial overlap of three proposed risk factors for zoonotic Ebola virus transmission in Uganda: modelled zoonotic niche, domestic pig distribution and high numbers of people living in extreme poverty.

areas correlating to the spatial overlap of risk for the resident population according to hypothesized, but as yet unproven risk factors. It does not indicate the actual likelihood of zoonotic spillover.

High-risk areas are found predominantly in the central and western parts of the country, with a few isolated areas in northern and eastern Uganda. All outbreak sites except for Gulu lie within clear risk areas. Considering Gulu lies as an outlier in the original zoonotic niche modelling data (Pigott et al., 2014) and is an area with virtually no pig production, this is not surprising. In addition, the cited human index cases for this outbreak does occur in an area with a large number of people living in extreme poverty and around a central forest reserve mosaic landscape, speculated to be high-risk environments for frequent human–animal contacts. One potential flaw in using the zoonotic niche data as an input layer for measuring risk in Uganda is that bat distributions used in the model are for three bat species that are found to have the most compelling evidence for carrying ZEBOV, an Ebola virus species which has not been reported from Uganda. Serological evidence for bat species carrying the strains found in Uganda is lacking. In this case, we are using the most comprehensive modelled zoonotic and ecological niche prediction data available. Future risk investigations using similar mapping methods could include (i) more collaborative organizational surveillance for reservoir bat species, other potential wildlife hosts and human populations in these hypothesized high-risk areas and (ii) analysis of specific ecological conditions such as high dependence on forest product utilization and certain biodiversity indicators, as well as behavioural associations with poverty, occupation and gender that may make risky zoonotic interactions and thus transmission of the virus to humans more likely.

Due to the sensitive nature of EVD and its tendency to create panic, disproportionate to the actual risk of infection, future research into the role pigs play in maintaining and transmitting Ebola virus needs to address:

1 The role pigs may play in Ebola virus transmission. The present data suggest they may be amplifying hosts, but not reservoir hosts. This suggests the conditions under which pigs become infection and the role they play in transmission may have many variables that will have to be elucidated.
2 Pig population dynamics as hosts of Ebola virus.
3 Risks factors to pig farming, specifically in relationship to bat and primate ecology and habitat.
4 Impact of Ebola virus on pig production, human health and livelihoods and food security.
5 Disease course and outcome of the different species of Ebola virus infection in pigs.
6 Communicating any risk of Ebola virus infection associated with pig production in ways that minimize adverse impacts on pig value chains, poverty and livelihoods.

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Author Contributions

All authors contributed extensively to the work presented in this manuscript. CA conducted the literature review and manuscript write-up. EM conducted the risk mapping and contributed to manuscript write-up. PO performed all the GIS analysis and created the different maps. KR and DG contributed to study design, supervised the research and gave feedback throughout the research and manuscript write-up.

Conflict of Interest

The author(s) declare no competing financial interests.

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Newly Discovered Ebola Virus Associated with Hemorrhagic Fever Outbreak in Uganda

Jonathan S. Towner[1], Tara K. Sealy[1], Marina L. Khristova[2], César G. Albariño[1], Sean Conlan[3], Serena A. Reeder[1], Phenix-Lan Quan[3], W. Ian Lipkin[3], Robert Downing[4], Jordan W. Tappero[4], Samuel Okware[5], Julius Lutwama[6], Barnabas Bakamutumaho[6], John Kayiwa[6], James A. Comer[1], Pierre E. Rollin[1], Thomas G. Ksiazek[1], Stuart T. Nichol[1]*

1 Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **2** Scientific Resources Program, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **3** Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, New York, United States of America, **4** Global AIDS Program, Centers for Disease Control and Prevention, Entebbe, Uganda, **5** Ministry of Health, Republic of Uganda, Kampala, Uganda, **6** Uganda Virus Research Institute, Entebbe, Uganda

Abstract

Over the past 30 years, *Zaire* and *Sudan ebolaviruses* have been responsible for large hemorrhagic fever (HF) outbreaks with case fatalities ranging from 53% to 90%, while a third species, *Côte d'Ivoire ebolavirus*, caused a single non-fatal HF case. In November 2007, HF cases were reported in Bundibugyo District, Western Uganda. Laboratory investigation of the initial 29 suspect-case blood specimens by classic methods (antigen capture, IgM and IgG ELISA) and a recently developed random-primed pyrosequencing approach quickly identified this to be an Ebola HF outbreak associated with a newly discovered ebolavirus species (*Bundibugyo ebolavirus*) distantly related to the *Côte d'Ivoire ebolavirus* found in western Africa. Due to the sequence divergence of this new virus relative to all previously recognized ebolaviruses, these findings have important implications for design of future diagnostic assays to monitor Ebola HF disease in humans and animals, and ongoing efforts to develop effective antivirals and vaccines.

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* E-mail: snichol@cdc.gov

Introduction

The family *Filoviridae* consists of two genera, *Marburgvirus* and *Ebolavirus*, which have likely evolved from a common ancestor [1]. The genus *Ebolavirus* is comprised of four species, *Zaire*, *Sudan*, *Reston* and *Côte d'Ivoire* (*Ivory Coast*) *ebolaviruses*, which have, with the exception of *Reston* and *Côte d'Ivoire ebolaviruses*, been associated with large hemorrhagic fever (HF) outbreaks in Africa with high case fatality (53–90%) [2]. Viruses of each species have genomes that are at least 30–40% divergent from one another, a level of diversity that presumably reflects differences in the ecologic niche they occupy and in their evolutionary history. Identification of the natural reservoir of ebolaviruses remains somewhat elusive, although recent PCR and antibody data suggest that three species of arboreal fruit bats may be carriers of *Zaire ebolavirus* [3]. No data has yet been published to suggest reservoirs for the *Sudan*, *Reston* and *Côte d'Ivoire ebolavirus* species. However, a cave-dwelling fruit bat has been recently implicated as a natural host for marburgvirus [4,5], supporting the hypothesis that different bat species may be the reservoir hosts for the various filoviruses.

Filovirus outbreaks are sporadic, sometimes interspersed by years or even decades of no apparent disease activity. The last new species of ebolavirus was discovered 14 years ago (1994), in Cote d'Ivoire (Ivory Coast), and involved a single non-fatal case, a veterinarian who performed an autopsy on an infected chimpanzee found in the Tai Forest [6]. No further disease reports have been associated with *Côte d'Ivoire ebolavirus*, in contrast to *Zaire* and *Sudan ebolaviruses* which have each caused multiple large outbreaks over the same time period. Here, we report the isolation and characterization of a new ebolavirus that was responsible for a large hemorrhagic fever outbreak in western Uganda. This new virus, proposed name *Bundibugyo ebolavirus*, is most closely related, albeit distantly, to *Côte d'Ivoire ebolavirus*, which was somewhat unexpected, given the large geographic distance between the two countries of origin.

Results/Discussion

In late November 2007 HF cases were reported in the townships of Bundibugyo and Kikyo in Bundibugyo District, Western Uganda (Figure 1A). A total of 29 blood samples were initially collected from suspect cases and sent in two air-transport shipments to the Centers for Disease Control and Prevention (CDC) for immediate testing. Evidence of acute ebolavirus infection was detected in eight specimens using a broadly reactive ebolavirus antigen capture assay known to cross-react with the different ebolavirus species [7] and an IgM capture assay based on *Zaire ebolavirus* reagents (Table 1). The Ugandan Ministry of Health was notified on November 28, 2007. These specimens were negative when initially tested with highly sensitive real-time RT-

Author Summary

In this report we describe a newly discovered ebolavirus species which caused a large hemorrhagic fever outbreak in western Uganda. The virus is genetically distinct, differing by more than 30% at the genome level from all other known ebolavirus species. The unique nature of this virus created challenges for traditional filovirus molecular based diagnostic assays and genome sequencing approaches. Instead, we quickly determined over 70% of the virus genome using a recently developed random-primed pyrosequencing approach that allowed the rapid development of a molecular detection assay that was deployed in the disease outbreak response. This draft sequence allowed easy completion of the whole genome sequence using a traditional primer walking approach and prompt confirmation that this virus represented a new ebolavirus species. Current efforts to design effective diagnostics, antivirals and vaccines will need to take into account the distinct nature of this important new member of the filovirus family.

PCR assays specific for all known *Zaire* and *Sudan ebolaviruses* and marburgviruses. However, further evidence of acute ebolavirus infection was obtained using a traditionally less sensitive (relative to the real-time RT-PCR assays) but more broadly reactive filovirus L gene-specific RT-PCR assay (1 specimen) (Table 1). Sequence analysis of the PCR fragment (400 bp of the virus L gene) revealed the reason for the initial failure of the real-time RT-PCR assays was that the sequence was distinct from the four known species of ebolavirus, although it was distantly related to *Cote d'Ivoire ebolavirus*. In total, 9 of 29 specimens showed evidence of ebolavirus infection, and all tests were negative for marburgvirus (data not shown).

Approximately 70% of the virus genome was rapidly (in less than 10 days) sequenced from total RNA extracted from a patient serum (#200706291) using a recently established metagenomics pyro-sequencing method developed at 454 Life Sciences which involves successive rounds of random DNA amplification [8]. Using the newly derived draft sequence, a real-time RT-PCR assay specific for the NP gene of this virus was quickly developed and evaluated. The assay was shown to have excellent sensitivity (Table 1), finding positive all the initial six samples that tested positive by either virus antigen capture (five specimens) or virus isolation assays (four specimens). The antigen-capture, IgM, IgG and newly designed real-time PCR assays were quickly transferred to the Uganda Virus Research Institute during the course of the outbreak to facilitate rapid identification and isolation of Ebola cases in the affected area for efficient control of the outbreak. The outbreak continued through late December, 2007, and resulted in 149 suspected cases and 37 deaths [9].

The entire genome sequence of this virus was completed using a classic primer walking sequencing approach on RNA from the reference virus isolate (#811250). The complete genome of the *Côte d'Ivoire ebolavirus* was not available, so it too was derived by a similar combination of random primed pyrosequencing and primer walking approaches. Acquisition of these sequences allowed for the first time the phylogenetic analysis of the complete genomes of representatives of all known species of Ebola and Marburg viruses. The analysis revealed that the newly discovered virus differed from the four existing ebolavirus species (Figure 1B), with approximately 32% nucleotide difference from even the closest relative, *Côte d'Ivoire ebolavirus* (Table 2). Similar complete genome divergence (35–45%) is seen between the previously characterized ebolavirus species. *Bundibugyo ebolavirus* is the proposed name for this newly identified species. This high level of genetic diversity translates to considerable amino acid differences in the encoded virus proteins. The virus surface glycoprotein, which is an important determinant of virus tropism and pathogenicity, differs between *Bundibugyo* and *Côte d'Ivoire* and *Zaire ebolaviruses* by over 27 and 35%, respectively at the amino acid level. Similarly, important virulence factors such as the immunosuppressive VP35 protein differ between *Bundibugyo* and *Côte d'Ivoire* and *Zaire ebolaviruses* by 23 and 25%, respectively. This extent of divergence will likely be reflected in significant antigenic and pathogenicity differences among these viruses.

Current human prototype ebolavirus vaccines include *Zaire* and *Sudan ebolaviruses* [10–12]. Cross-protection studies will need to be done to assess whether vaccine designs will need to incorporate the *Bundibugyo ebolavirus*. The unique nature of this virus has other implications too, including screening of potential antivirals and pathogenicity studies. Retrospective analysis of case description, epidemiologic and laboratory data from the Bundibugyo outbreak are still ongoing, but it is clear that the case fatality (~36%) associated with *Bundibugyo ebolavirus* infection is lower than that observed for *Zaire ebolavirus* (approx. 80–90%) and *Sudan ebolavirus* (approximately 50–55%) [2]. Studies in non-human primates need to be performed to compare the pathogenicity of these viruses. This investigation also highlights the power of molecular detection and characterization tools to quickly identify new pathogens, while providing a cautionary note regarding sole dependence on molecular techniques such as real-time PCR assays for detection of novel agents in biodefense or emerging disease surveillance programs.

Materials and Methods

Ebolavirus detection and virus isolation

Several diagnostic techniques were used for each sample: (i) antigen capture, IgG, and IgM assays were performed as previously described [7,13] (ii) virus isolation attempts were performed on Vero E6 cells [14] and monitored for 14 days; (iii) RNA was extracted and tested for *Zaire* [15] and *Sudan ebolavirus* and marburgvirus [4] using real-time quantitative RT-PCR assays designed to detect all known strains of each respective virus species [the primers/probe for the *Sudan ebolavirus* assay were EboSudBMG 1(+) 5′-GCC ATG GIT TCA GGT TTG AG-3′, EboSudBMG 1(−) 5′-GGT IAC ATT GGG CAA CAA TTC A and EboSudBMG Probe 5′FAM-AC GGT GCA CAT TCT CCT TTT CTC GGA-BHQ1]; (iv) the conventional RT-PCR was performed with the filo A/B primer set as previously described [16] using Superscript III (Invitrogen) according to the manufacturer's instructions. The specimen 200706291 was selected as the reference sample for further sequence analysis.

Genome sequencing

Pyro-sequencing was carried out utilizing the approach developed by 454 Life Sciences, and the method described by Cox-Foster et al., [8]. Subsequent virus whole genome primer walking was performed as previously described [17] but using the primers specific for *Bundibugyo ebolavirus* RT-PCR amplification. In total, the entire virus genome was amplified in six overlapping RT-PCR fragments (all primers listed 5′ to 3′): fragment A (predicted size 2.7 kb) was amplified using forward-GTGAGACAAAGAATCATTCCTG with reverse-CATCAATTGCTCAGAGATCCACC; fragment B (predicted size 3.0 kb) was amplified using forward-CCAACAACACTGCATGTAAGT with reverse-AGGTCGCGTTAATCTTCATC; fragment C (predicted size 3.5 kb)

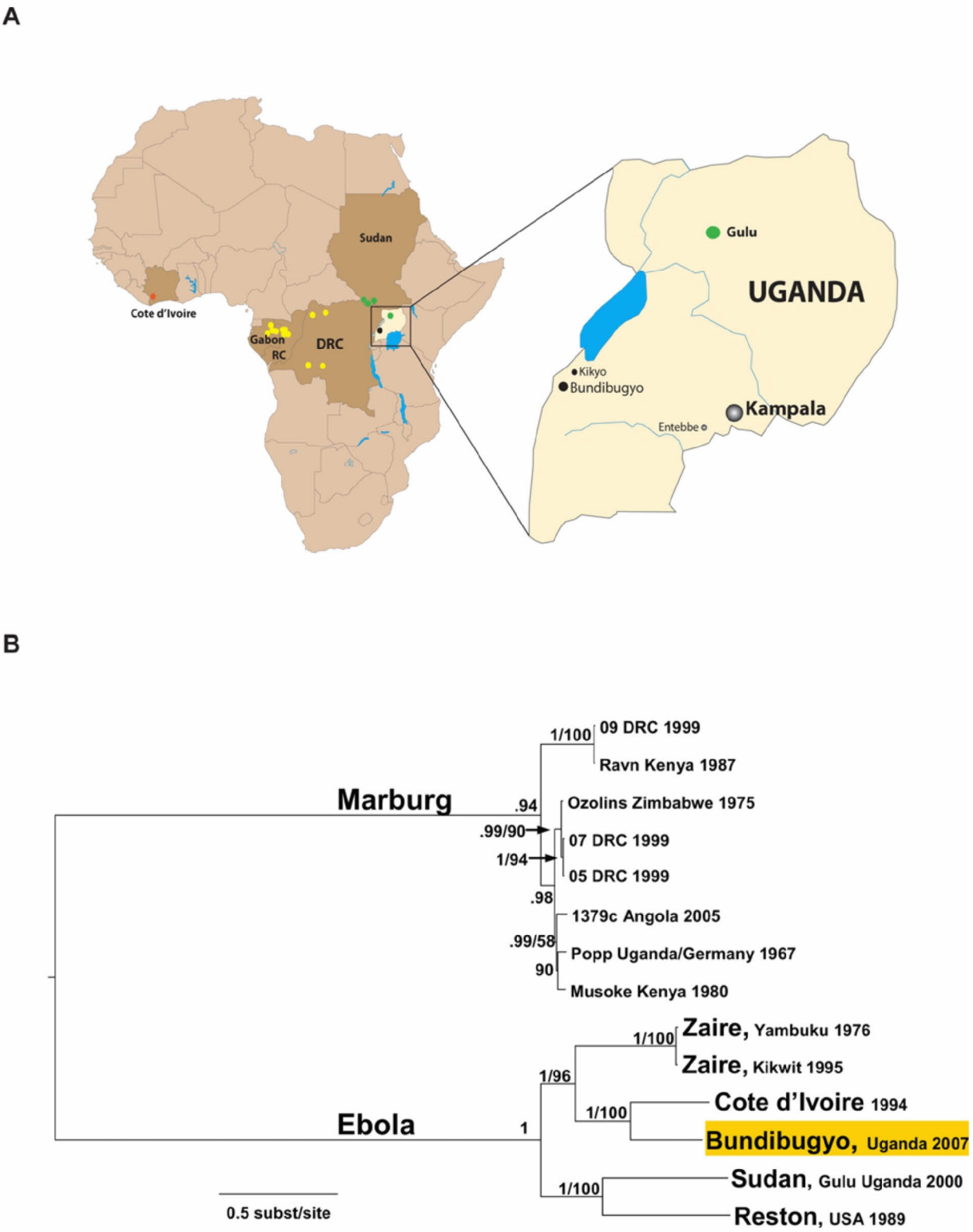

Figure 1. Geographic locations of Ebola HF outbreaks and phylogenetic relationships of representative filoviruses. (A) Map of Africa showing the sites of all known ebolavirus outbreaks denoted by colored circles for *Zaire ebolavirus* (yellow), *Sudan ebolavirus* (green), and *Côte d'Ivoire ebolavirus* (red). The expanded map of Uganda shows the location of the communities of Bundibugyo and Kikyo (black circles) in western Uganda, the site of the recent outbreak of *Bundibugyo ebolavirus*. Also shown on the Uganda map are the cities of Kampala (capital), Entebbe (international airport) and Gulu (the site of an outbreak of *Sudan ebolavirus* in 2000, the largest known Ebola HF outbreak on record). (B) Phylogenetic tree comparing full-length genomes of ebolavirus and marburgvirus by Bayesian analysis. Posterior probabilities greater than 0.5 and maximum likelihood bootstrap values greater than 50 are indicated at the nodes.
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Table 1. Ebolavirus diagnostic results of initial 29 specimens obtained from Bundibugyo District with numerical specimen numbers assigned.

Sample No.	RT-PCR	Ag	IgM	IgG	Virus Isolation	Q-RT-PCR	Ct
200706288	neg	neg	neg	neg	neg	neg	40
200706289	neg	neg	neg	neg	neg	neg	40
200706290	neg	neg	neg	neg	neg	neg	40
200706291*	**Pos**	**Pos**	neg	neg	**Pos**	**Pos**	**23.64**
200706292	neg	neg	neg	neg	neg	neg	40
200706293	neg	neg	neg	neg	neg	neg	40
200706294	neg	neg	neg	neg	neg	neg	40
200706295	neg	neg	neg	neg	neg	neg	40
200706296	neg	neg	**Pos**	**Pos**	neg	neg	40
200706297	neg	neg	**Pos**	**Pos**	neg	neg	40
200706298	neg	**Pos**	**Pos**	**Pos**	neg	**Pos**	**34.83**
200706299	neg	neg	**Pos**	**Pos**	neg	neg	40
200706300	neg	neg	neg	neg	neg	neg	40
200706301	neg	neg	neg	neg	neg	neg	40
200706302	neg	**Pos**	**Pos**	neg	neg	**Pos**	**35.01**
200706303	neg	neg	neg	neg	neg	neg	40
200706304	neg	neg	neg	neg	**Pos**	**Pos**	**38.18**
200706305	neg	neg	neg	neg	neg	neg	40
200706306	neg	neg	neg	neg	neg	neg	40
200706307	neg	neg	neg	neg	neg	neg	40
200706320	ND	**Pos**	neg	neg	**Pos**	**Pos**	**30.24**
200706321	ND	neg	neg	neg	neg	neg	40
200706322	ND	neg	neg	neg	neg	neg	40
200706323	ND	neg	neg	neg	neg	neg	40
200706324	ND	neg	neg	neg	neg	neg	40
200706325	ND	neg	neg	neg	neg	neg	40
200706326	ND	neg	neg	neg	neg	neg	40
200706327	ND	**Pos**	neg	neg	**Pos**	**Pos**	**34.41**
200706328	ND	neg	neg	neg	neg	neg	40

RT-PCR refers to results obtained from conventional PCR using the broadly reactive Filo A/B primers [16]. Ag, IgM, and IgG refer to results from ELISA-based assays [7,13] with *Zaire ebolavirus* reagents while virus isolation refers to culture attempts on Vero E6 cells [14]. Q-RT-PCR refers to results obtained using the optimized *Bundibugyo ebolavirus* specific real-time RT-PCR assay with cycle threshold (Ct) values of positive (Pos) samples indicated in the far right column.

*Specimen # 200706291 is the clinical sample from which prototype isolate #811250 was obtained.

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was amplified using forward-GATGGTTGAGTTACTTTCCGG with reverse-GTCTTGAGTCATCAATGCCC; fragment D (predicted size 3.1 kb) was amplified using forward-CCACCAGCACCAAAGGAC with reverse-CTATCGGCAATGTAACTATTGG; fragment E (predicted size 3.4 kb) was amplified using forward-GCCGTTGTAGAGGACACAC with reverse-CACATTAAATTGTTCTAACATGCAAG and fragment F (predicted size 3.5 kb) was amplified using forward-CCTAGGTTATTTAGAAGGGACTA with reverse-GGT AGA TGT ATT GAC AGC AAT ATC.

The exact 5′ and 3′ ends of *Bundibugyo ebolavirus* were determined by 3′ RACE from virus RNA extracted from virus infected Vero E6 cell monolayers using TriPure isolation reagent. RNAs were then polyadenylated in vitro using A-Plus poly(A) polymerase tailing kit (Epicenter Biotechnologies) following the manufacturer's instructions and then purified using an RNeasy kit (Qiagen) following standard protocols. Ten microliters of in vitro polyadenylated RNA were added as template in RT-PCR reactions, using SuperScript III One-Step RT-PCR system with Platinum *Taq* High Fidelity (Invitrogen) following the manufacturer's protocol. Two parallel RT-PCR reactions using the oligo(dT)-containing 3′RACE-AP primer (Invitrogen) mixed with 1 of 2 viral specific primers, Ebo-U 692(−) ACAAAAAGCTATCTGCACTAT and Ebo-U18269(+) CTCAGAAGCAAAATTAATGG, generated ~700 nt long fragments containing the 3′ ends of either genomic and antigenomic RNAs. The resulting RT-PCR products were analyzed by agarose electrophoresis, and DNA bands of the correct sizes were purified using QIAquick Gel Extraction Kit (Qiagen) and sequenced using standard protocols (ABI).

The nucleotide sequence of the *Côte d'Ivoire ebolavirus* isolate RNA was initially determined using the exact same pyrosequencing strategy as that used for *Bundibugyo ebolavirus* described above. This method generated sequence for approximately 70% of the entire genome. This draft sequence was then used to design a whole genome primer walking strategy for filling any gaps and

Table 2. Identity (percent) matrix based on comparisons of full-length genome sequences.

	Zaire '95	Sudan '00	CdI '94	Bundi '07	Reston '89
Zaire '76	98.8	57.7	63.0	63.2	58.1
Zaire '95		57.7	63.1	63.3	58.1
Sudan '00			57.7	57.7	60.9
CdI '94				68.3	57.5
Bundi '07					57.6

The genomic sequences in the analysis are *Zaire ebolaviruses* 1976 (Genbank accession number NC_002549) and 1995 (Genbank accession number AY354458), *Sudan ebolavirus* 2000 (Genbank accession number NC_006432), *Cote d'Ivoire (CdI) ebolavirus* 1994 (Genbank accession number FJ217162), *Reston ebolavirus* 1989 (Genbank accession number NC_004161), and *Bundibugyo (Bundi) ebolavirus* 2007 (Genbank accession number FJ217161).
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confirming the initial sequence. The following *Côte d'Ivoire ebolavirus*-specific primers were used to generate RT-PCR fragments, designated A–F, as follows: Fragment A (predicted size 3.0 kb) was amplified using forward-GTGTGCGAATAACTATGAGGAAG and reverse-GTCTGTGCAATGTTGATGAAGG; Fragment B (predicted size 3.2 kb) was amplified using forward-CATGAAAACCACACTCAACAAC and reverse-GTTGCCTTAATCTTCATCAAGTTC; Fragment C (predicted size 3.0 kb) was amplified using forward-GGCTATAATGAATTTCCTCCAG and reverse-CAAGTGTATTTGTGGTCCTAGC; fragment D (predicted size 3.5 kb) was amplified using forward-GCTGGAATAGGAATCACAGG and reverse-CGGTAGTCTACAGTTCTTTAG; fragment E (predicted size 4.0 kb) was amplified using forward-GACAAAGAGATTAGATTAGCTATAG and reverse-GTAATGAGAAGGTGTCATTTGG; fragment F (predicted size 2.9 kb) was amplified using forward-CACGACTTAGTTGGACAATTGG and reverse-CAGACACTAATTAGATCTGGAAG; fragment G (predicted size 1.3 kb) was amplified using forward-CGGACACACAAAAAGAAWRAA and reverse-CGTTCTTGACCTTAGCAGTTC; and fragment H (predicted size 2.5 kb) was amplified using forward-GCACTATAAGCTCGATGAAGTC and reverse-TGGACACACAAAAARGARAA. A gap in the sequence contig was located between fragments C and D and this was resolved using the following primers to generate a predicted fragment of 1.5 kb: forward-CTGAGAGGATCCAGAAGAAAG and reverse-GTGTAAGCGTTGATATACCTCC. The terminal ~20 nucleotides of the sequence were not experimentally determined but were inferred by comparing with the other known Ebola genome sequences.

Bundibugyo ebolavirus real-time RT-PCR assay

The primers and probe used in the *Bundibugyo ebolavirus* specific Q-RT-PCR assay were as follows: EboU965(+): 5′-GAGAAAAGGCCTGTCTGGAGAA-3′, EboU1039(−): 5′-TCGGGTATTGAATCAGACCTTGTT-3′ and EboU989 Prb: 5′Fam-TTCAACGACAAATCCAAGTGCACGCA-3′BHQ1. Q-RT-PCR reactions were set up using Superscript III One-Step Q-RT-PCR (Invitrogen) according to the manufacturer's instructions and run for 40 cycles with a 58°C annealing temperature.

Phylogenetic analysis

Modeltest 3.7 [18] was used to examine 56 models of nucleotide substitution to determine the model most appropriate for the data. The General Time Reversible model incorporating invariant sites and a gamma distribution (GTR+I+G) was selected using the Akaike Information Criterion (AIC). Nucleotide frequencies were A = 0.3278, C = 0.2101, G = 0.1832, T = 0.2789, the proportion of invariant sites = 0.1412, and the gamma shape parameter = 1.0593. A maximum likelihood analysis was subsequently performed in PAUP*4.0b10 [19] using the GTR+I+G model parameters. Bootstrap support values were used to assess topological support and were calculated based on 1,000 pseudoreplicates [20].

In addition, a Bayesian phylogenetic analysis was conducted in MrBayes 3.2 [21] using the GTR+I+G model of nucleotide substitution. Two simultaneous analyses, each with four Markov chains, were run for 5,000,000 generations sampling every 100 generations. Prior to termination of the run, the AWTY module was used to assess Markov Chain Monte Carlo convergence to ensure that the length of the analysis was sufficient [22]. Trees generated before the stabilization of the likelihood scores were discarded (burn in = 40), and the remaining trees were used to construct a consensus tree. Nodal support was assessed by posterior probability values (≥95 = statistical support).

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Author Contributions

Conceived and designed the experiments: JST TKS MLK CGA STN. Performed the experiments: JST TKS MLK CGA SAR BB JK JAC. Analyzed the data: JST TKS MLK CGA SC SAR BB JK JAC PER TGK STN. Contributed reagents/materials/analysis tools: JST TKS MLK CGA SC SAR PLQ WIL RD JWT SO JL BB JK JAC PER TGK STN. Wrote the paper: JST TKS CGA SAR STN.

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From: Baric, Ralph S <rbaric@email.unc.edu>
To: Anthony, Simon J. <sja2127@cumc.columbia.edu>;Kirsten Gilardi <kvgilardi@ucdavis.edu>;Jonna Mazet <jkmazet@ucdavis.edu>
Sent: 3/21/2017 12:34:31 PM
Subject: RE: mBio press release

OK! Press is good!

From: Anthony, Simon J. [mailto:sja2127@cumc.columbia.edu]
Sent: Tuesday, March 21, 2017 3:25 PM
To: Kirsten Gilardi; Jonna Mazet; Baric, Ralph S
Subject: mBio press release

Dear all -

Just spoke to a science writer who is doing a piece for mBio on the MERS-like paper. I recommended she (Karen Blum) contact each of you to get insights for into the sample collection (Kirsten), PREDICT (Jonna) and the reverse genetics (Ralph). Hope that is ok.

Cheers
S.

Simon J Anthony, D.Phil
Assistant Professor, Department of Epidemiology
Center for Infection and Immunity, Columbia University

722 West 168th Street, 17th Floor
NY, NY, 10032

Email: sja2127@cumc.columbia.edu
Mobile: 760-500-4639
Office: 212-342-0558

From: Andrew Clements <aclements@usaid.gov>
Sent: Thu, 23 Mar 2017 21:08:21 +0100
Subject: Re: Monkeypox outbreak in ROC
To: Jonna Mazet <jkmazet@ucdavis.edu>
Cc: PREDICTMGT <predictmgt@usaid.gov>, "predict@ucdavis.edu" <predict@ucdavis.edu>

thanks. every little bit helps. :)

On Thu, Mar 23, 2017 at 5:25 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Quick update on low-cost Predict support,
J

---------- Forwarded message ----------
From: **Karen Saylors** <ksaylors@metabiota.com>
Date: Thu, Mar 23, 2017 at 8:59 AM
Subject: Re: Monkeypox outbreak in ROC
To: "predict-outbreak@ucdavis.edu" <predict-outbreak@ucdavis.edu>, Jonna Mazet <jkmazet@ucdavis.edu>, Brian Bird <bhbird@ucdavis.edu>
Cc: David John Wolking <djwolking@ucdavis.edu>, Eddy Rubin <erubin@metabiota.com>

Good morning.
I got follow up from the Country Coordinator in ROC today. In the meeting on Monday, it sounds like there was an urgent request for biosecurity material contributions to the DGLM (General Office of Disease Control of MOH). Today our team donated a modest amount of biosecurity equipment, specifically the following supplies:
Latex gloves (Fisherbrand): 4 packs of 100
Respiratory masks (N95): 2 packs of 20
Protective glasses: 10 pieces
Protective jacket (complete): 5 pieces
Antimicrobial handsoap (Liquid): 1 box

Apparently, the MOH response team was very pleased, especially since PREDICT was one of the first partners to do so.
Thanks,
Karen

From: Karen Saylors <ksaylors@metabiota.com>
Date: Wednesday, March 22, 2017 at 8:13 AM
To: "predict-outbreak@ucdavis.edu" <predict-outbreak@ucdavis.edu>, Jonna Mazet <jkmazet@ucdavis.edu>, Brian Bird <bhbird@ucdavis.edu>
Cc: David John Wolking <djwolking@ucdavis.edu>, Eddy Rubin <erubin@metabiota.com>
Subject: Re: Monkeypox outbreak in ROC

Hello everyone.
I would like to provide you with an update from our Country Coordinator today on the MPX outbreak.
At Monday's MOH DGMLM meeting on March 20, the working group reviewed and validated the epidemic report dated March 9, 2017 (attached). The Country Coordinator will send us the most updated report as soon as it is corrected and disseminated.

This report is in French but to summarize salient points:
—up through March 8, there had been 20 suspected cases and 3 deaths in the administrative districts of Bétou, Enyellé, Dongou et Impfondo.
—they've provided an epi age and sex breakdown of 10 of these cases on p.2. Apparently, information on the the 10 cases from Manfouété (Dongou) was inadequate to be able to ascertain age/sex, so they are not included.
—On p 4 we have a map of where suspected and confirmed MPX cases were located, as well as deaths/survivors.
—On the last page, you have a list of needs for the response effort, but it is not clear whether certain materials have been requested/provided by specific organizations yet.

A joint mission "CDC - Experts Congo" traveled today to the department of Likouala. The DGELM has requested "biosecurity material" from PREDICT, which I assume means PPE, but I have requested a specific list of what they are requesting, which I hope to have and share tomorrow.

Thanks,
Karen

From: <[REDACTED]> on behalf of Jonna Mazet <jkmazet@ucdavis.edu>
Date: Friday, March 17, 2017 at 2:51 PM
To: Karen Saylors <ksaylors@metabiota.com>
Cc: "preduict-outbreak@ucdavis.edu" <preduict-outbreak@ucdavis.edu>, David John Wolking <djwolking@ucdavis.edu>, Eddy Rubin <erubin@metabiota.com>
Subject: Re: Monkeypox outbreak in ROC

Thanks, Karen.
Keep us posted if you receive more info or requests for support.
Jonna

On Fri, Mar 17, 2017 at 11:43 AM, Karen Saylors <ksaylors@metabiota.com> wrote:

Good morning.

I received some follow up from our Country Coordinator this morning regarding MPX in Congo. The MoH General Director, Head of Epi and Disease Control, has called a meeting with country partners for Monday, March 20th.

Mostly Congolese entities have been convoked, as well as UNICEF and UN, but not projects like PREDICT specifically. CC will follow up with action items on Monday.

Please find attached and translated below the Technical Update that Col Bagamboula provided to MOH Epi and Disease Control Department today, in his role as Military Health Technical Director, not as PREDICT CC:

In the Likouala Department, the first cases of the disease were reported in 2003, and since then, training took place in our country with the support of the US CDC for the recognition and surveillance of this disease.

To date, partial investigations in the district of Enyelle and Betou, as well as cases from the Manfouete village in the district of Dongou and those notified in Impfondo, equate to twenty (20) for the number of people suffering from this disease. Patients ages varies between 4 and 40 years. Of these 20 cases, three deaths have been verified: two deaths in the camp of Dignonga (in Manfouete, district of Dongou) and one in Mouale (district of Enyelle). Difficult access to certain areas of the Department limits investigation capacity and thus knowledge of the situation in remote areas. In parallel, an outbreak of measles has also been declared in the same Department.

In response to these two epidemic threats, the Directorate General of Epidemiology and Disease Control (DGELM), the World Health Organization (WHO) and the Office of the High Commissioner for Refugees (UNHCR) Techniques at the central level, sent delegates to support the department in investigating and preparing the response: a national response plan against Monkeypox and measles was developed. The international NGO "Land Without Borders", which is a UNHCR medical partner, has provided personnel for the investigation and notification of cases in Enyellé, Bétou and Dongou.

Formal instructions were given to the Chief of the Military Zone of Defense No. 6 Impfondo, who must ensure the awareness and protection of law enforcement officers and their families against these two outbreaks. The latter works in collaboration with the Departmental Director of Health of Likouala.

For your information, the following measures have been taken to reduce the mortality and morbidity associated with Monkeypox and measles in Likouala:

- Coordination: set up and operationalize a local epidemic management committee

- Epidemiological surveillance: reporting and investigating 100% of suspect cases that meet operational definitions;

- Responsive vaccination against measles: vaccinate at least 95% of the target population in all districts;

- Communication and community mobilization: informing at least 90% of the population about the two threats and the prevention measures;

- Case management: to take charge, according to national protocols, of 100% of cases examined in health centers;

- Control of infection: apply general hygiene measures in all health facilities;

- Logistical support: provide logistical support to contain the infection;

- Post-epidemic surveillance: take all necessary measures to end the epidemic to learn lessons and avoid a resurgence of cases.

--

Andrew Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
E-mail: aclements@usaid.gov

For more information on USAID's Emerging Pandemic Threats program, see: http://www.usaid.gov/ept2

From: Catherine Machalaba <machalaba@ecohealthalliance.org>
To: "William B. Karesh" <karesh@ecohealthalliance.org>, Woutrina A Smith <wasmith@ucdavis.edu>
Cc: Jonna Mazet <jkmazet@ucdavis.edu>, Chris Johnson <ckjohnson@ucdavis.edu>
Subject: Re: One Health case studies and messaging
Sent: Wed, 29 Mar 2017 20:39:50 +0000

Thank you so much for thinking of us, Woutrina! As Billy mentioned, we are closely coordinated with them and I am indeed a member, listed on their website ;) I presented on PREDICT at their January meeting and saw presentations on the case studies they have assembled (and shared our One Health in Action case study booklet and Lessons Learned from PREDICT documents).
I agree there are definitely some synergies between our two groups, and it was great to have them involved in the One Health Economics workshop and I hope they will also take the methods forward that we developed and continue to refine them. They are applying assessment to an interesting range of issues, including obesity and animal welfare, so there's not always immediate tie-in for the PREDICT scope of One Health assessment to inform practices and policies to reduce viral evolution, spillover, amplification and spread. There was also interest from some of their members in applying their assessment criteria to PREDICT, but honestly, I am quite concerned about the time it would require for PREDICT teams to provide the information - which is in large part oriented to assessing the level of One Health-ness (and still in development and not quite policy-relevant at this point). My view is that we can continue to share information and learn from each others' approaches and case studies and apply the assessment criteria that makes sense for our targeted goals.

I am definitely keeping a close eye on their work for specific areas of alignment with our PREDICT mandate and am also involved in the development of their handbook- I'll look forward to sharing that resource as it will be a nice complement to the tools we are developing in PREDICT. Many thanks!

Kind regards,
Catherine

Catherine Machalaba, MPH
Health and Policy Program Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4472 (direct)
[redacted]
1.212.380.4465 (fax)
www.ecohealthalliance.org

Science Officer, Future Earth oneHEALTH Project

Chair, Veterinary Public Health Special Primary Interest Group, American Public Health Association

Program Officer, IUCN SSC Wildlife Health Specialist Group

On Mar 29, 2017, at 3:17 PM, William B. Karesh <karesh@ecohealthalliance.org> wrote:

Hi Wout,
Please do not forward this message.

Catherine has been going to the NEOH meetings and coordinating with Barbara and the others in that group. Catherine negotiated a deal with them to exchange case studies that we can use in future PREDICT materials. We are a bit ahead of them in products so their materials will hopefully come in this year or next.

They didn't have any funds to support the workshop we did with the World Bank recently but we had several of their key leadership attend and listed them as a co-sponsor of the workshop to help with getting wider EU buy-in for our process. And, yes, Jacob is active with that group.

BK

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001 USA

+1.212.380.4463 (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Mar 29, 2017, at 1:47 PM, Woutrina A Smith <wasmith@ucdavis.edu> wrote:

FYI below on a One Health group interested in case studies, messaging, etc. Are any of you already involved with them or should I reach out? Woutrina

Begin forwarded message:

From: "Munoz,Olga" <omunoz@ufl.edu>
Subject: nice meeting you
Date: March 29, 2017 at 8:12:20 AM PDT
To: "wasmith@ucdavis.edu" <wasmith@ucdavis.edu>

Hi Woutrina,

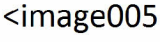
It was nice meeting you and Wendy and thank you once more for accepting our invitation to give the talk. I will send you the link to the recording as soon as it becomes available. It is a pleasure to meet people willing to collaborate and working through a One Health philosophy, I believe it is essential for the different One Health Centers to cooperate in order to send unified and strong messages to the community, continue building a solid base to evidence and push the use of a One Health approach in research and development and, of course, to gather more fruitful results.
This is the link to the cost action I was telling you about: http://neoh.onehealthglobal.net/. There are no EcoHealth Alliance members there, it turns out I was imagining a network in my head through Jakob Zinsstag, as he frequently writes about EcoHealth =) It is a mostly European network, so I am sure they would appreciate collaboration from the other side of the Atlantic. Here is Barbara Haesler's email:bhaesler@rvc.ac.uk .

Please let us know next time you are in Gainesville and let's stay in touch,

Have a nice flight back to California,

Kind regards,

Olga

Olga Muñoz, DVM, MSc
Research Assistant, One Health Center of Excellence
Emerging Pathogens Institute
University of Florida
32611, Gainesville, Florida
Tel: (352) 294-8589



From:	Kevin Olival, PhD <olival@ecohealthalliance.org>
To:	predict-surveillance@ucdavis.edu <predict-surveillance@ucdavis.edu>;Christine Kreuder Johnson <ckjohnson@ucdavis.edu>
CC:	Catherine Machalaba <machalaba@ecohealthalliance.org>;William B. Karesh" <karesh@ecohealthalliance.org>;Jonna Mazet <jkmazet@ucdavis.edu>;Megan M Doyle <mmdoyle@ucdavis.edu>;Evan Eskew <eskew@ecohealthalliance.org>;Peter Daszak <daszak@ecohealthalliance.org>
Sent:	4/5/2017 9:12:26 AM
Subject:	Re: [predict-surveillance] PREDICT surveillance call March 16th, 2017 @ 10am PT/1pm ET

Dear Chris and all,

Thank you all for the excellent feedback we received on the 16 March PREDICT surveillance call re: the P1 viral curve analyses. I'm attaching a revised version of the .html document we discussed (complete with interactive plots and tables) that incorporates the changes you suggested.

These changes include:

- Viral accumulation curves now represent data solely from PCR testing (no deep sequencing or serology)
- A plot focusing on data that tested for P-2 priority viral families only
- Plots that separate out testing for a given P-2 priority viral family by testing protocol, e.g. two CoV protocols
- Scrolling over host curves in all plots now show the breadth of viral family level testing conducted on that host aggregation (species or genus)
- Color coding by host Order is now consistent across all interactive plots (blue = bats, orange=primates, red=rodents)

Please let us know if you have any further suggestions to improve this and make it most useful.

Cheers,
Kevin and the M&A team

PREDICT-1 Global Viral Accumulation Analyses

EcoHealth Alliance M&A Team, Code Drafted by Evan Eskew and Cale Basaraba

DRAFT for review on P-2 Surveillance Call, 16 March 2017 UPDATED

Viral accumulation by host species

This report provides an overview of viral accumulation analyses conducted on PREDICT-1 data using the EIDITH R package and iNEXT R package. Overall, we hope that these analyses, or updated versions of them, will be of value to P-2 surveillance teams to help prioritize species targets, prioritize archived specimens for further testing, and determine species-specific sample size targets based on past data.

This first interactive plot shows viral accumulation curves for individual host species from all P-1 global data. To summarize the method in broad terms, using viral incidence data (the observed PREDICT data), we want to see the rates at which viral diversity (i.e., viral species richness) is expected to accumulate as we test more and more specimens from each host species. Host species that have no viral detections, or just one observed virus, are uninformative using this method and have been excluded here. Thus, the plot shows only sampled species that have at least two unique viruses detected over the course of P-1. In addition, the EIDITH database here has been subset down to remove serology and high throughput sequencing test results. Therefore, results represent PCR tests only.

A few notes on interpretting and using the interactive plot:

- Each viral accumulation curve is plotted as a series of points. Triangles represent observed, interpolated data, and these symbols extend up to the total observed sample size for a given host species (shown as a square). Circles then represent extrapolated values of predicted viral diversity (modeled using iNEXT). iNEXT does not recommend extending diversity estimates beyond twice the observed sample size. Thus, extrapolated values stop at twice the sample size for every host species.
- Viral accumulation curves are plotted in color according to host taxonomic order.
- The "Zoom" feature allows you to focus on a subset of the data. Press the "Reset axes" button (the little house) to return to the original plot.
- Mousing over a data point will display information about host species, order, data type (observed vs. extrapolated), and the viral families that were tested for.
- The search tool at the top of the plot allows you to filter to particular host species of interest. The dropdown menu displays all host species in order of number of viruses observed. This filtering tool is searchable. Since species' common names are included in the labels, you can search for those as well. For example, "house rat" and "Rattus rattus" work equally well.
- You can select multiple species to compare viral curves side by side.

IMPORTANT: Note that this plot does not account for differences in testing across specimens (i.e., viral diversity estimates will be lower in species that were only tested for a subset of viruses). In addition, keep in mind that only 77.6 percent of animal_ids in the database have associated binomial nomenclature, so there are some specimens not represented here if their taxonomic information is not detailed enough.

Host Species

Searchable table of viral diversity estimates and specimen testing data, PREDICT-1

Predicted viral diversity information from iNEXT could be used to help us identify species and specimens to prioritize for further viral testing. The following table allows us to easily search for that information. Some notes on the table:

- Each row of the table represents information on a particular species ("binomial") in a given country.
- Following taxonomic and geographic information, the next few columns of the table correspond to country-specific testing information, including:
  - The number of animals collected ("n_animals")
  - The number of specimens collected ("n_specimens")
  - The number of viral tests run ("n_tests")
  - The number of viral groups tested for ("n_viral_test_types")
  - The number of animals tested ("n_animals_tested")
  - The number of specimens tested ("n_specimens_tested")
  - The percentage of specimens tested ("pct_specimens_tested")
  - A yes/no variable indicating a small number of specimens (< 20) having been tested ("small_sample?")
  - The number of viruses observed in country ("n_viruses_obs_in_country")
- The next columns then correspond to global-level information on a species, including:
  - The number of viruses observed across all countries ("n_viruses_obs_global")
  - The number of viruses predicted at twice the observed global sample size ("n_viruses_est_2x"). This estimate corresponds with the last viral accumulation curve point for a given host species
  - The asymptotic estimate for the number of viruses ("n_viruses_est_asy")
  - The lower confidence limit for the asymptotic viral diversity estimate ("n_viruses_est_asy_lower")
  - The upper confidence limit for the asymptotic viral diversity estimate ("n_viruses_est_asy_upper")
  - The difference between the asymptotic viral diversity estimate and the observed number of viruses ("n_viruses_est_remaining_global")
  - The above metric expressed as a percentage ("pct_viruses_est_remaining_global")
- Any column in the table can be sorted using the arrow buttons in the column headings. In addition, the table is searchable, enabling easy filtering to country- or species-specific data.

It may be of particular interest to focus viral testing on species that are predicted to have high absolute values of viral diversity remaining to be discovered (high "n_viruses_est_remaining_global") or those for which a large portion of the species' viral diversity likely remains unobserved (high "pct_viruses_est_remaining_global").

Keep in mind that these analyses are not necessarily well controlled in the sense that different specimens may have been tested for drastically different sets of viruses. One way to control for differences in viral testing is to simply subset down to look only at specimens that have been tested for a given viral group and do viral accumulation curve analyses group by group. For example, it may make sense to subset down and only look at pathogen groups that are a priority for PREDICT-2 sampling. Shown below are plots showing only specimens that have been tested for all five priority viral groups and then those showing specimens tested for each viral group individually. In addition, within viral families that were tested for, we can further subset down to particular testing protocols that were used, since these may affect viral recovery. Note that since multiple viral species were not recovered from any single host species for the Filoviruses, Flaviviruses, or Influenzas, data from these families are not plotted individually.

All PREDICT-2 Priority Viral Groups (Coronaviruses, Filoviruses, Flaviviruses, Influenzas, Paramyxoviruses) - All Protocols

Host Species

Coronaviruses Only - Quan Protocol

Host Species

Coronaviruses Only - Watanabe Protocol

Host Species

Paramyxoviruses Only - Tong Pol Gene Protocol

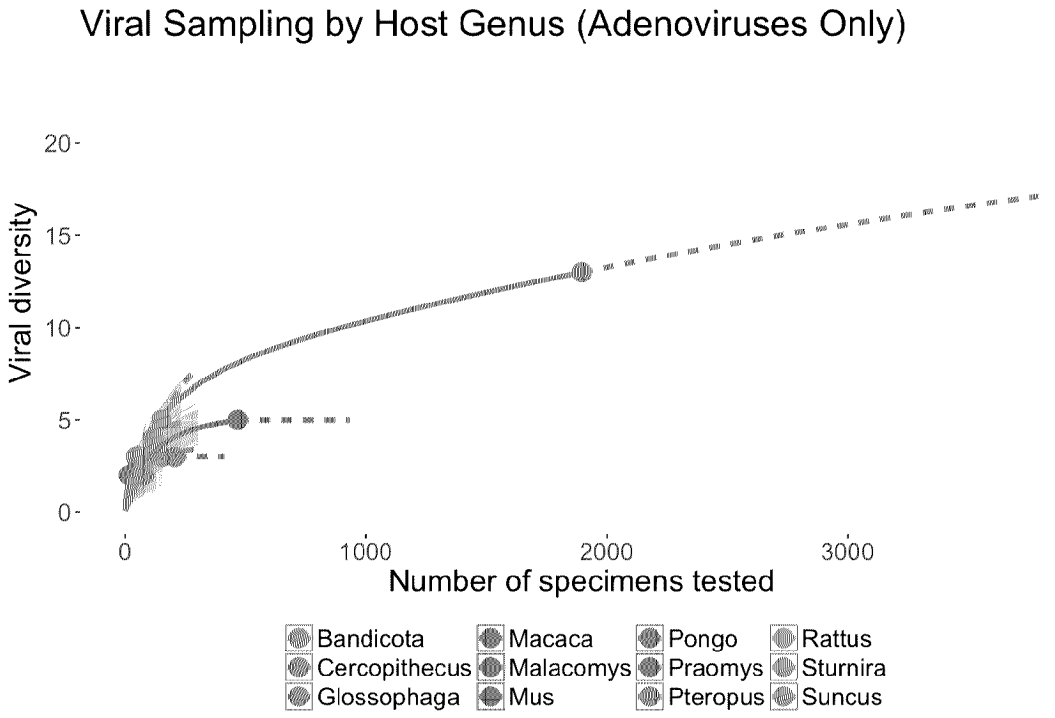
Host Species

Viral accumulation by host genus

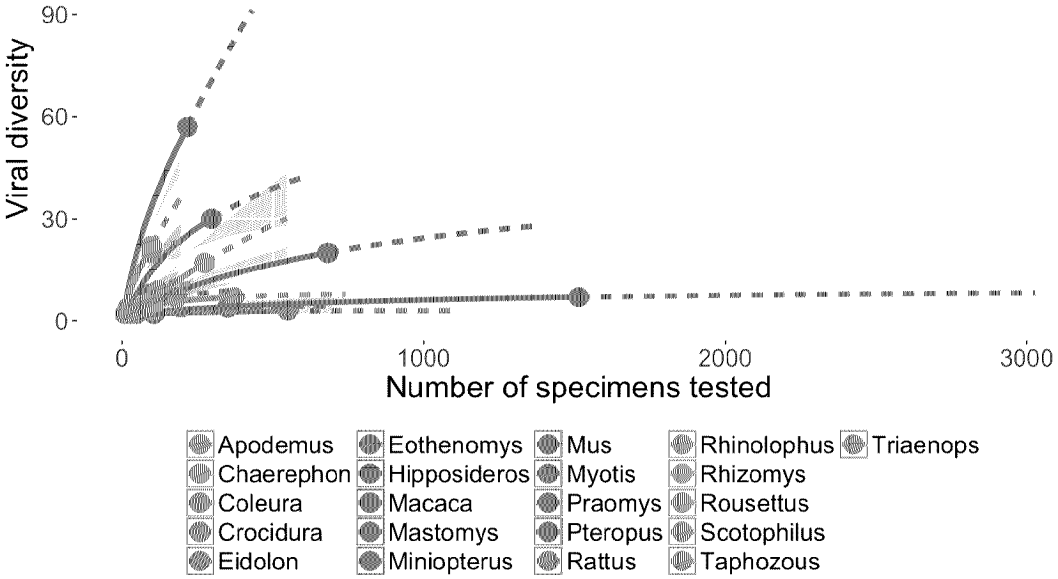
In addition to summarizing by host species, it may also make sense to summarize to some other taxonomic unit. For example, rather than looking at the species level, we could take a coarser approach and summarize to host genus.

Host Genus

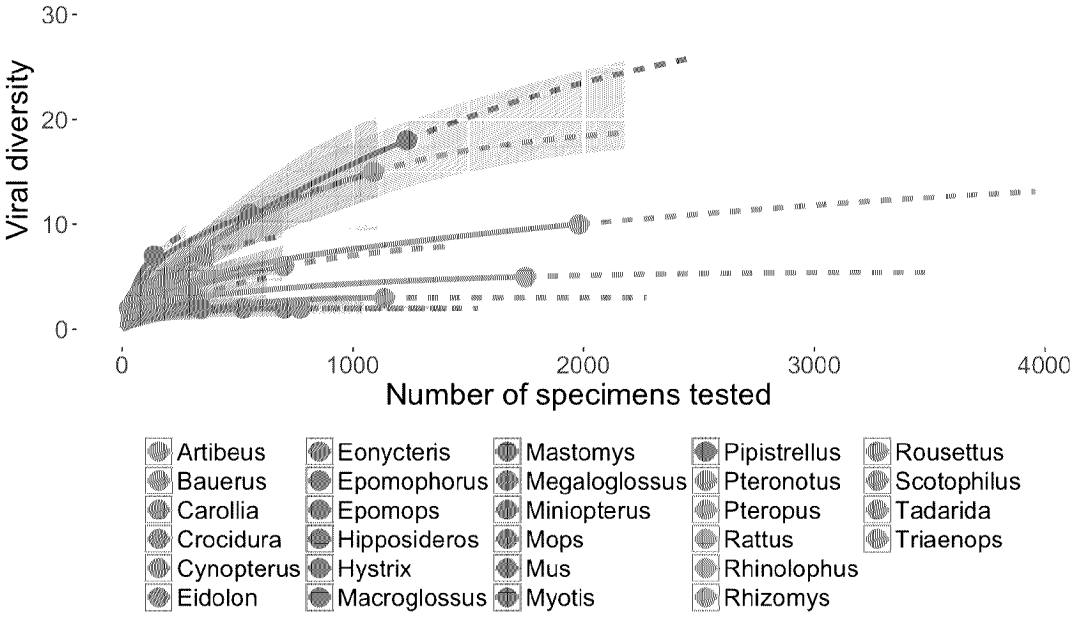
As before, in order to control for differnces in viral testing among specimens, we should subset the data. Shown below are viral families for which multiple host genuses were tested and at least 5 distinct viruses were observed in at least one genus (irrespective of testing protocol). Here only static plots are shown, and in general, controlling for differences in viral testing results in less drastic differences in viral accumulation among host taxonomic groups.



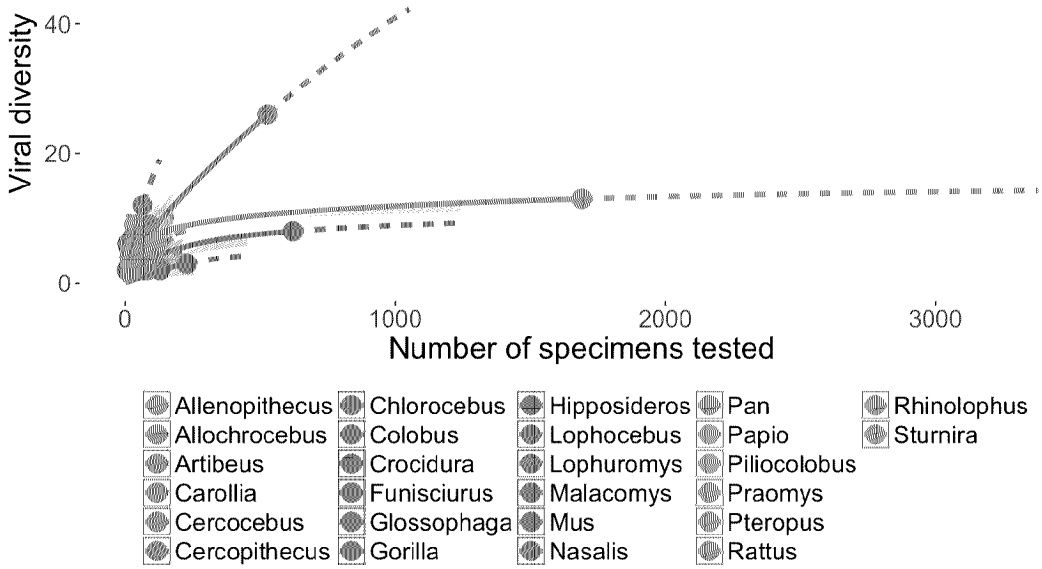
Viral Sampling by Host Genus (Astroviruses Only)



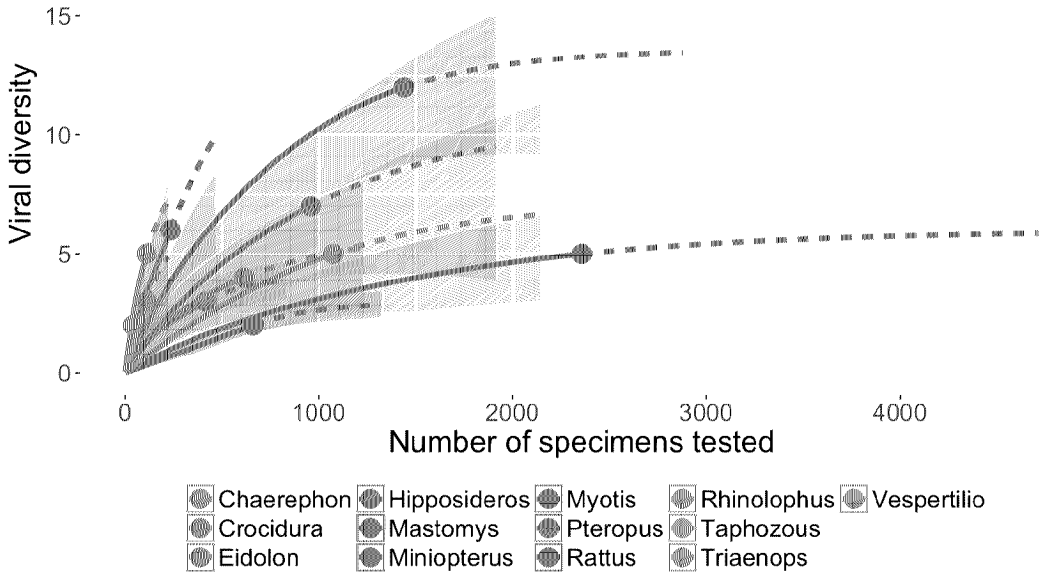
Viral Sampling by Host Genus (Coronaviruses Only)

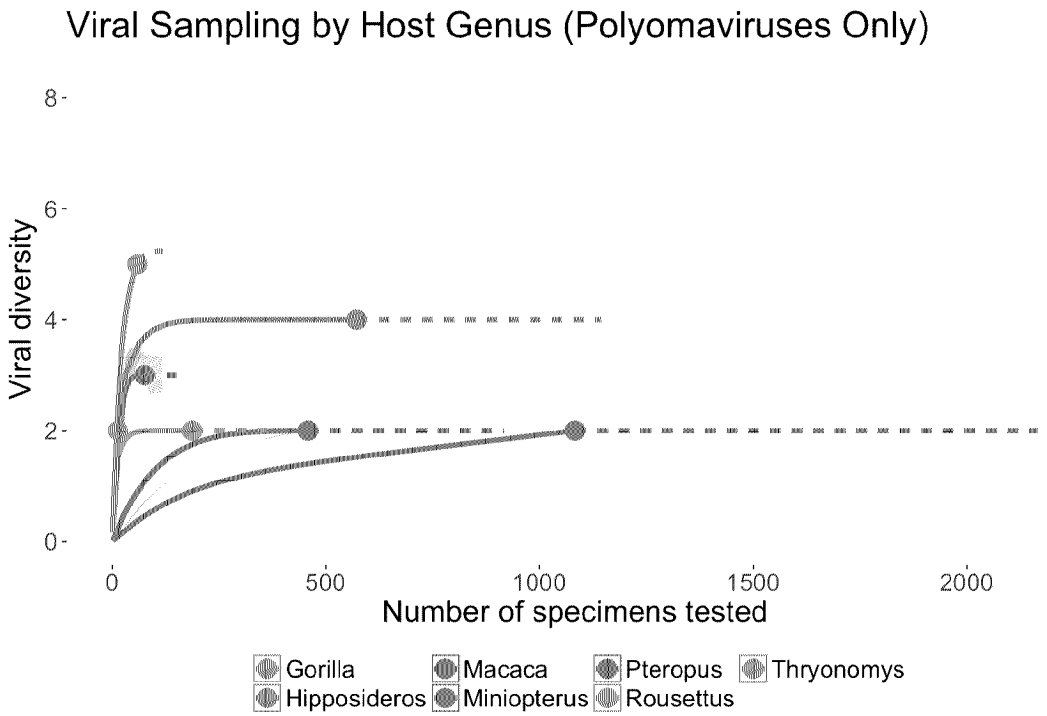


Viral Sampling by Host Genus (Herpesviruses Only)



Viral Sampling by Host Genus (Paramyxoviruses Only)





Kevin J. Olival, PhD
Associate Vice President for Research

USAID PREDICT-2 Modeling & Analytics Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4478 (direct)
REDACTED (mobile)
1.212.380.4465 (fax)
www.ecohealthalliance.org

On Mar 15, 2017, at 10:57 AM, Kevin Olival, PhD <olival@ecohealthalliance.org> wrote:

Dear all,
Chris asked me to share the attached .html document with you all in advance of tomorrow's surveillance call. It's a large file (8.2MB), but you should be able to open it easily with any web browser (e.g. Google Chrome, Safari, etc).

The document summarizes preliminary analyses from the Modeling & Analytics (M&A) team to estimate viral diversity per host from global PREDICT-1 data. The first couple of plots and table are searchable and interactive, so we encourage you to play around with these before the call if you have time. On the call, Evan Eskew (PREDICT M&A team member) and I will walk you through what we've done (+ some of the caveats with these approaches), and get suggestions on how to improve these analyses to be of the most value for surveillance and the project overall. Looking forward to the call tomorrow!

Cheers,
Kevin

<viral_accumulation_for_surveillance.html>

Kevin J. Olival, PhD
Associate Vice President for Research
PREDICT-2 Modeling & Analytics Coordinator

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4478 (direct)
REDACTED (mobile)
1.212.380.4465 (fax)
@nycbat (twitter)
www.ecohealthalliance.org

On Mar 14, 2017, at 4:08 PM, Christine Kreuder Johnson <ckjohnson@ucdavis.edu> wrote:

Hi Team,
Our next surveillance call is this Thurs, March 16th, 2017 @ 10am PT/1pm ET.
Call in REDACTED
International Dial-in number: REDACTED (toll charges apply)

Please note we have a special request for information from countries in Africa affected by the recent change in FAO plans –
We are seeking country level information on whether PREDICT would want to undertake livestock sampling and testing given that FAO is no longer supporting this activity.
Our guidance is that PREDICT should only add livestock if this is an essential aspect of our surveillance plan/inferences AND we have the capacity to take this on, given all other deliverables that we have in the time remaining on the project.
We're unsure if there's the potential for additional funds to do this, but if you would like to take on livestock sampling and testing, we need an estimate of the projected # of samples, and the amount of additional funds that would be needed for the remainder of the project.
Please add this information directly into the attached spreadsheet in the yellow highlighted column and note cost (for both field and lab activities). We'll need this information by next Thursday 3/23 and we can all clarify further on surveillance call.

Draft Agenda

- Viral discovery curves for P1 data (Kevin; attachment to follow)
- Preparation for new rodent blood sampling techniques (pending IACUC approval)
- EIDITH data entry progress
- Activities tracker (see attached)
- Impact of FAO changes on field activities
- Africa field activities/updates

Please send any additional agenda items for our upcoming call.
Also attaching notes here from our last call on March 2nd.
Warm regards and talk soon,
Chris

Christine Kreuder Johnson, VMD, PhD
Professor of Epidemiology and Ecosystem Health
Global Surveillance Coordinator, Emerging Pandemic Threats PREDICT Project
One Health Institute
VM3B 1089 Veterinary Medicine Drive
One Health Institute
School of Veterinary Medicine
University of California
Davis, California 95618
+1.530.752.1238

<3.2.2017 surveillance call notes.docx><PREDICT country activities tracker March 3 2017.xlsx>

From: Patrick Dawson <dawson@ecohealthalliance.org>
Sent: Thu, 13 Apr 2017 11:30:05 -0400
To: David J Wolking <djwolking@ucdavis.edu>
Cc: "predict@ucdavis.edu" <predict@ucdavis.edu>, "William B. Karesh" <karesh@ecohealthalliance.org>, Amanda Andre <amanda.andre@ecohealthalliance.org>, Emily Hagan <hagan@ecohealthalliance.org>, Leilani Francisco <francisco@ecohealthalliance.org>
Subject: [predict] Re: Jordan IRB Pre-Submission Materials

Hi David,
Perfect, I'll change that and we'll submit to JUST's IRB.

Thank you!
Patrick

On Thu, Apr 13, 2017 at 11:28 AM, David J Wolking <djwolking@ucdavis.edu> wrote:

> Hey Patrick,
> Looks good to me, one small change to the UCD IRB reference number. You can just use "804522" without the suffix (e.g., "-15"), which refers to a country modification. We are already on 21 now and climbing.

Good luck with the local review.

David

On Thu, Apr 13, 2017 at 8:19 AM, Patrick Dawson <dawson@ecohealthalliance.org> wrote:

> Hi David,
> Thank you so much - I have addressed your comments:
>
> - UCD IRB approved study number referenced & included as an appendix
> - Added all human questionnaire modules to the submission packet
> - Specified that consent form, info sheet, and questionnaire will be translated into the local language, Arabic
> - Added language to "Risks and Benefits" from "Privacy and Confidentiality of Subjects" about results sharing
>
> Please let me know if you have any other questions.

If this looks good to you, we will move forward with the local IRB at JUST. Thanks again!

Best regards,
Patrick

On Mon, Apr 10, 2017 at 11:09 AM, David J Wolking <djwolking@ucdavis.edu> wrote:

> Patrick,
> Apologies for the delay. Everything looks good, a few comments in the main protocol document for you to review (e.g., translations of docs?, use of modules from the human questionnaire?, mentions of results sharing in the protocol itself, etc....).

If you do plan to include the UCD approved master IRB with the packet like we did with Egypt then I don't think this will raise any eyebrows here.

Let me know if you have any questions,

David

On Tue, Mar 28, 2017 at 9:06 PM, Patrick Dawson <dawson@ecohealthalliance.org> wrote:

Hi David,
Thank you for your reply -- We would like to get started as soon as possible so we can fulfill Jordan's Y3 work plan of 200 individuals by September. We expect the local IRB to take approximately 3 weeks once we submit, and we would like to get started by late May/early June if at all possible. We have tentative plans to conduct trainings in Jordan later this month or during May.

Thank you so much,
Patrick

On Tue, Mar 28, 2017 at 7:14 PM, David J Wolking <djwolking@ucdavis.edu> wrote:

Thanks Patrick much appreciated. I'm buried with reporting right now. What's the timeline for Jordan submission (optimal date to get this in locally, expected review/turnaround time, and your best bet for when you'd like to launch activities?). That info will help me prioritize review.
Cheers,

D

On Thu, Mar 23, 2017 at 4:27 PM, Patrick Dawson <dawson@ecohealthalliance.org> wrote:

Dear David,
I am writing to submit materials for IRB pre-submission approval for Jordan. Adhering to the pre-submission checklist available on EIDITH, the following items are included:

Submission Checklist:

- [ ] 1. Submitted a bulleted list of changes made to all global document(s) to predict@ucdavis.edu.
- [ ] 2. Shared the country plan developed on the PREDICT global protocol template (using Track Changes as descried in the instructions) with predict@ucdavis.edu.
- [ ] 3. Used the most recent version of the Master Protocol documents (available at http://eidith.org/Resources/PREDICTIRBProtocols.aspx) to develop in-country materials.
- [ ] 4. Submitted English language versions of the country protocol, written consent form, verbal consent form, introductory script, and human questionnaire (Word.doc preferred) to predict@ucdavis.edu. Provided English language versions of any printed advertising materials to predict@ucdavis.edu.
- [ ] 5. Provided in-country IRB submission requirements (in copy or by web link, or if not in English via a document explaining the requirements) to predict@ucdavis.edu.
- [ ] 6. Clearly listed all Country Coordinators, Human Surveillance Coordinators, Global Leads, and key US-based staff involved in the study in the country protocol personnel list. These individuals will require CITI training.

Please let me know if anything else is needed. Thank you!

Best regards,
Patrick

--

Patrick Dawson, MPH
Research Scientist and PREDICT Country Liaison

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.646.868.4712 (direct)
REDACTED (mobile)
1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

--

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From:	Andrew Clements <aclements@usaid.gov>
To:	Dennis Carroll <dcarroll@usaid.gov>;Amalhin Shek <ashek@usaid.gov>;Lindsay Parish <lparish@usaid.gov>;Alisa Pereira <apereira@usaid.gov>;Daniel Schar (RDMA/OPH) <dSchar@usaid.gov>;Angela Wang <awang@usaid.gov>;Sudarat Damrongwatanapokin (RDMA/OPH) <sDamrongwatanapokin@usaid.gov>;eadelman@usaid.gov <eadelman@usaid.gov>;William Karesh <Karesh@ecohealthalliance.org>;Jonna Mazet <jkmazet@ucdavis.edu>;Christine Kreuder Johnson <ckjohnson@ucdavis.edu>
Sent:	4/18/2017 1:22:09 AM
Subject:	Fwd: PRO/AH/EDR> Avian influenza, human (44): China, H7N9, updates, pandemic potential

FYI. See NPR story on pandemic potential of H7N9

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

Begin forwarded message:

From: promed-edr@promedmail.org
Date: April 18, 2017 at 4:01:22 AM GMT+2
To: promed-post@promedmail.org, promed-edr-post@promedmail.org, promed-ahead-post@promedmail.org
Subject: PRO/AH/EDR> Avian influenza, human (44): China, H7N9, updates, pandemic potential
Reply-To: promedNOREPLY@promedmail.org

AVIAN INFLUENZA, HUMAN (44): CHINA, H7N9, UPDATES, PANDEMIC POTENTIAL
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

In this Update;
[1] Beijing Fatal Case
[2] Shandong
[3] Henan Cases
[4] Sichuan Case
[5] Tianjin Case
[6] Hebei Case
[7] Article on Sichuan Cases
[8] Avian and human influenza virus compatible sialic acid receptors in bats
[9] Avian influenza A/H7N9 potential threat

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[1] Beijing Fatal Case
Date: Fri 14 Apr 2017, 1:45 PM
Source: ECNS [edited]

<http://www.ecns.cn/2017/04-14/253449.shtml>

One person has died in the Chinese capital Beijing as a result of the newest strain of bird flu in the country, the Municipal Commission of Health and Family Planning said [Thu 13 Apr 2017].

In a statement, the commission said 2 new people were diagnosed with H7N9 bird flu in Beijing. One patient died last [Sat 8 Apr 2017] and another is in the hospital receiving treatment. No other information was released other than the fact that both people had direct contact with live poultry.

Data from the commission show 5 people have been infected the H7N9 bird flu strain so far this year [2017] in Beijing. Authorities warned locals to stay away from live poultry, and to make sure food is completely cooked before consumption.

[Byline: CGTN, editor: Li Yan]

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Communicated by:
ProMED-mail
<promed@promedmail.org>

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[2] Shandong
Date: Thu 13 Apr 2017
Source: FIC (Flu Information Centre/Flu in China) [edited]
<http://www.flu.org.cn/en/news_detail?action=ql&uid=MjI0OA&pd=YXRsbXBw&newsId=19252>

A human H7N9 AIV case confirmed in Qingdao city of Shandong province on [Tue 11 Apr 2017]. The 60-year-old male patient lived in Huangdao district of Qingdao city.

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<promed@promedmail.org>

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[3] Henan Cases
Date: Fri 14 Apr 2017
Source: FIC (Flu Information Centre/Flu in China) [edited]
<http://www.flu.org.cn/en/news-19263.html>

Henan province reported 2 human H7N9 AIV cases between [Sat 8 and Fri 14 Apr 2017]; 1 case in Nanyang city and the other in Pingdingshan city.

The 45-year-old female patient, surname Li is a resident of Nanzhao

county of Nanyang city. She was in serious condition and remained in hospital for treatment.

The 54-year-old male patient, surname Deng is a resident of Xinhua district of Pingdingshan city. He was also in serious condition and remained in hospital for treatment.

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[4] Sichuan Case
Date: Sat 15 Apr 2017
Source: FIC (Flu Information Centre/Flu in China) [edited]
<http://www.flu.org.cn/en/news-19266.html>

A human H7N9 AIV case reported in Chuanshan district of Suining city of Sichuan province on [Fri 7 Apr 2017]. The 48-year-old male patient, surname Teng, lived in Chuanshan district of Suining city, was in stable condition and remained in hospital for treatment. No close contacts have shown ILI symptoms so far.

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[5] Tianjin Case
Date: Fri 14 Apr 2017
Source: FIC (Flu Information Centre/Flu in China) [edited]
<http://www.flu.org.cn/en/news_detail?action=ql&uid=MjI0OA&pd=YXRsbXBw&newsId=19258>

Tianjin reported a human H7N9 AIV case on [Thu 13 Apr 2017]. The 58-year-old female patient, surname Zhou, lived in Wuqing district of Tianjin city. She was admitted into designated hospital for severe pneumonia and her sample tested positive for H7N9 nucleic acid by Tianjin CDC. All close contacts of the patient are under close medical monitoring and none has showed ILI symptoms so far.

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<promed@promedmail.org>

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[6] Hebei Case
Date: Sat 15 Apr 2017
Source: FIC (Flu Information Centre/Flu in China) [edited]

<http://www.flu.org.cn/en/news-19269.html>

According to the Langfang CDC, a human H7N9 AIV case was confirmed in Dacheng county of Langfang city. The 69-year-old male patient, surname Liu, lived in Nanzhaofu town of Dacheng county of Langfang city, had poultry contact history before onset of the symptoms and remained in Langfang people´s hospital for treatment.

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ProMED-mail
<promed@promedmail.org>

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[7] Article on Sichuan Cases
Date: Thu 13 Apr 2017
Source: FIC (Flu Information Centre/Flu in China) [edited]
<http://www.flu.org.cn/en/article_detail?action=ql&uid=MjI0OA&pd=YXRsbXBw&articleId=11480>

Article titled 'Severely ill human infection with avian influenza A (H7N9) virus firstly identified in Sichuan province: 6 cases report and clinical analysis'. Chinese Journal of Respiratory and Critical Care M. by XIA Hongtao,et al.

Abstract

Objective: Since the 1st case of avian influenza A (H7N9) virus infection in humans identified in Suining, Sichuan province on [25 Jan 2017], there were other 5 severely ill patients confirmed in the following 3 weeks. It is urgent to find out the common clinical characters of these patients, so that to make sure the optimal ways for early diagnosis and treatment for H7N9 virus infection in community hospitals or primary hospitals as soon as possible.
Methods: The early symptoms, the data of early laboratory findings, the early imaging study, and the early process of diagnosis and treatment of these 6 patients were collected and analyzed.
Results: All 6 patients had high fever, dry cough, hypocalcemia, and hypophosphatemia, with advanced CT image lesions manifested as consolidation and ground glass opacity in bilateral lower lung lobes. Some patients had typically leukopenia, lymphopenia, thrombocytopenia. And most of them had a history of direct exposure to live poultry before complaining of flu-like syndromes. However, the flu could not be effectively controlled by routine anti-infection [treatment].
Conclusion: The human infection with H7N9 virus can be identified early combining the epidemiology of live poultry exposure, the symptoms of high fever, dry cough, dramatical leukopenia, lymphopenia, thrombocytopenia, the typical CT image, and the rapidly worsening clinical condition.

Full article available at
<http://www.flu.org.cn/scn/article_detail.asp?articleId=11473>.

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ProMED-mail
<promed@promedmail.org>

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[8] Avian and human influenza virus compatible sialic acid receptors in bats
Date: Thu 6 Apr 2017
Source: Nature, Scientific reports [edited]
<https://www.nature.com/articles/s41598-017-00793-6>

Citation: Chothe SK, Bhushan G, Nissly RH, et al. Avian and human influenza virus compatible sialic acid receptors in little brown bats. Sci Rep. 2017 Apr 6;7(1):660. doi: 10.1038/s41598-017-00793-6.
Abstract

Influenza A viruses (IAVs) continue to threaten animal and human health globally. Bats are asymptomatic reservoirs for many zoonotic viruses. Recent reports of 2 novel IAVs in fruit bats and serological evidence of avian influenza virus (AIV) H9 infection in frugivorous bats raise questions about the role of bats in IAV epidemiology. IAVs bind to sialic acid (SA) receptors on host cells, and it is widely believed that hosts expressing both SA alpha 2,3-Gal and SA alpha 2,6-Gal receptors could facilitate genetic reassortment of avian and human IAVs. We found abundant co-expression of both avian (SA alpha 2,3-Gal) and human (SA alpha 2,6-Gal) type SA receptors in little brown bats (LBBs) that were compatible with avian and human IAV binding. This 1st ever study of IAV receptors in a bat species suggest that LBBs, a widely-distributed bat species in North America, could potentially be co-infected with avian and human IAVs, facilitating the emergence of zoonotic strains.

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<promed@promedmail.org>

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[9] Avian influenza A/H7N9 potential threat
Date: Tue 11 Apr 2017, 5:06 AM ET
Source: National Public Radio (NPR) report [edited]
<http://www.npr.org/sections/goatsandsoda/2017/04/11/523271148/why-chinese-scientists-are-more-worried-than-ever-about-bird-flu>

At a research lab on top of a forested hill overlooking Hong Kong, scientists are growing viruses. They first drill tiny holes into an egg before inoculating it with avian influenza to observe how the virus behaves.

This lab at Hong Kong University is at the world's forefront of our understanding of H7N9, a deadly strain of the bird flu that has killed more people this season -- 162 from September [2016] up to [1 Mar 2017] than in any single season since when it was 1st discovered in humans 4 years ago. That worries lab director Guan Yi. But what disturbs him more is how fast this strain is evolving. "We're trying our best, but we still can't control this virus," says Guan. "It's too late for us to eradicate it."

Guan is one of the world's leading virologists. He has held some of the worst in his hands: H1N5, H1N1 and SARS. His work on Severe Acute Respiratory Syndrome, or SARS, in 2003 led to the successful identification of its infectious source from live animal markets and helped China's government control the virus that had killed hundreds, avoiding a 2nd outbreak. He has now moved on to avian influenza.

Guan's office, which has a view of the lush hills and blue waters of Hong Kong Harbor, is decorated with ceramic figurines of ducks, geese and chickens. His adjacent lab is full of tissue samples of the birds -- and of deceased humans -- all of whom have perished from H7N9.

The fowl samples -- along with live birds -- arrive from a network of scientists who, each week, purchase birds at poultry markets throughout southern China. Back in December [2016], Guan and his colleague Zhu Huachen began noticing something strange about them. "Some of the birds ... they will die within a day," says Zhu.

A lab assistant at the University of Hong Kong Center of Influenza Research will inoculate eggs with bird flu to track how the virus behaves.

The birds that were quick to die of H7N9 were all chickens. This was a surprise, because chickens normally live with the virus in what's known as a low pathogenic state -- they carry the virus but don't die from it and have a low capacity to spread it. Guan and his team discovered the H7N9 strain had mutated into a new form that kills chickens even more quickly. "Ten years ago, H7N9 was less lethal," says Guan. "Now it's become deadlier in chickens. Before it barely affected chickens. Now many are dying. Our research shows it can kill all the chickens in our lab within 24 hours. If this latest mutation isn't stopped, more will die."

Guan says this is very bad news for a global poultry industry that's worth hundreds of billions of dollars, and he says China's government is already looking into vaccinating chickens. What worries Guan more, though, is that H7N9 has proved an ability to mutate quickly. There's no evidence that the virus has become more deadly in people. But already, in the rare cases when humans catch it from birds, more than a 3rd of them die.

Currently, the virus hasn't been known to spread easily among humans, but Guan fears a future mutation could. "Based on my 20 years of studying H7N9 -- the virus itself as well as how the government

handles it -- I'm pessimistic," says Guan, shaking his head. "I think this virus poses the greatest threat to humanity than any other in the past 100 years."

Guan's choice of 100 years is deliberate. Next year will mark the 100th anniversary of what was known as the Spanish flu, the most devastating epidemic recorded in history. As World War I drew to a close, the influenza of 1918 killed between 20 million and 50 million people, all dead from a flu that originated in birds. He says it's not a stretch to envision another global pandemic. "Today, science is more advanced, we have vaccines and it's easy to diagnose," says Guan. "On the other hand, it now takes hours to spread new viruses all over the world."

Keiji Fukuda, former global director of the World Health Organization's Influenza Program, is also concerned. "We are now able to make vaccines and analyze things faster, but at the same time, the movement of people and animals is faster. There's a balance in those things. Some are helpful, some aren't. Everything's moving more quickly, and it's a shifting thing."

Fukuda, who now teaches at Hong Kong University's school of public health, says H7N9's ability to mutate from low pathogenic to highly pathogenic -- deadly and infectious -- in chickens disturbs him. "It makes us queasy," Fukuda says. "Because it's a very visible way to see these viruses as restless. Some of these changes are dead-end, but some are not. And this genetic mutation is not. It's becoming more lethal for poultry. For people? We're not sure."

What's worse, says Guan, is that new mutations of the bird flu virus are typically discovered in China, a country with scores of small poultry farms run by farmers who aren't well-educated about the threat of bird flu and who often hide evidence of infected birds to protect their bottom line. "Farmers are scared of losing money. I know how they think -- I'm from a rural part of China. And that's why I'm not optimistic about this."

Guan says there are a few encouraging signs. Big cities like Shanghai have quickly shut down their live poultry markets when human cases are on the rise. Guan says preventing the next global pandemic will depend on how well the governments of individual countries collaborate. That, he says, is a different challenge altogether.

[Byline: Rob Schmitz]

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<promed@promedmail.org>

[Lessons learnt from the challenges posed during the control and prevention of avian influenza A/H5N1 and A/H1N1 outbreaks are rich enough to build further national, regional, and global health

security. Even though timeliness and completeness of disease reporting can be demanding and have improved with the advances of information and communication technologies, availability of adequate laboratory diagnostic capacity at national and regional levels is a continuous challenge.

The training of sufficient workforce of rapid response teams with field epidemiology skills can support the preparedness and response activities for avian influenza as well as other public health threats.
- Mod.UBA

A HealthMap/ProMED-mail map can be accessed at:
<http://healthmap.org/promed/p/155>.]

[See Also:
Avian influenza, human (43): China, H7N9
http://promedmail.org/post/20170412.4962707
Avian influenza, human (42): China, H7N9
http://promedmail.org/post/20170409.4959020
Avian influenza, human (41): China, H7N9, WHO
http://promedmail.org/post/20170406.4945768
Avian influenza, human (39): China, H7N9
http://promedmail.org/post/20170331.4935953
Avian influenza, human (38): China, H7N9
http://promedmail.org/post/20170327.4926547
Avian influenza, human (37): China, H7N9, WHO update, control, genetics http://promedmail.org/post/20170324.4923674
Avian influenza, human (36): China, H7N9
http://promedmail.org/post/20170320.4910882
Avian influenza, human (35): China, H7N9, WHO updates
http://promedmail.org/post/20170317.4905430
Avian influenza, human (34): China (JX,CQ) H7N9
http://promedmail.org/post/20170316.4898107
Avian influenza, human (32): China, H7N9
http://promedmail.org/post/20170310.4890695
Avian influenza, human (30): China, H7N9
http://promedmail.org/post/20170307.4885433
Avian influenza, human (29): China (SH, Mainland), H7N9, WHO assessment http://promedmail.org/post/20170304.4878682
Avian influenza, human (28): China (GX) Taiwan, H7N9, mutations
http://promedmail.org/post/20170302.4874114
Avian influenza, human (27): Egypt (Fayoum) H5N1, RFI
http://promedmail.org/post/20170227.4866795
Avian influenza, human (26): China (HE) H7N9
http://promedmail.org/post/20170227.4866739
Avian influenza, human (25): China (SD, GX), H7N9
http://promedmail.org/post/20170225.4863940
Avian influenza, human (24): China (JX), H7N9, control measures
http://promedmail.org/post/20170224.4861044
Avian influenza, human (23): China, Taiwan, H7N9, WHO, genetic mutations http://promedmail.org/post/20170223.4858369
Avian influenza, human (22): China (GX, SD), H7N9, WHO updates, vaccine http://promedmail.org/post/20170222.4852285

Avian influenza, human (21): China (GZ) H7N9
http://promedmail.org/post/20170219.4849594
Avian influenza, human (20): China (SC, YN, BJ), H7N9, death toll
http://promedmail.org/post/20170215.4841682
Avian influenza, human (10): Indonesia (LA) RFI
http://promedmail.org/post/20170123.4785841
Avian influenza, human (01): China (JX), H7N9
http://promedmail.org/post/20170102.4736553
2016

Avian influenza, human (72): China (HK) H7N9, fatal
http://promedmail.org/post/20161229.4727495
Avian influenza, human (68): China, H7N9
http://promedmail.org/post/20161218.4705001
Avian influenza, human (67): WHO, H5N6, H7N9, risk assessment
http://promedmail.org/post/20161212.4689184
Avian influenza, human (66): China (GD) H7N9
http://promedmail.org/post/20161210.4689085
Avian influenza, human (65): China (HN) H5N6
http://promedmail.org/post/20161123.4646005
Avian influenza, human (64): China (ZJ,JS) H7N9
http://promedmail.org/post/20161114.4624064
Avian influenza, human (60): China (JX) H9N2
http://promedmail.org/post/20160912.4481431
Avian influenza, human (58): China, H7N9, update, WHO
http://promedmail.org/post/20160820.4422893
Avian influenza, human (57): China, H7N9, WHO update
http://promedmail.org/post/20160727.4370565
Avian influenza, human (55): China (mainland) H7N9, fatal
http://promedmail.org/post/20160722.4362599
Avian influenza, human (54): China, H7N9, WHO
http://promedmail.org/post/20160714.4343947
Avian influenza, human (53): China (LN) H7N9, fatal
http://promedmail.org/post/20160710.4332434
Avian influenza, human (52): WHO, human-animal interface
http://promedmail.org/post/20160625.4308644
Avian influenza, human (48): China (HN) H5N6
http://promedmail.org/post/20160610.4275291
Avian influenza, human (45): WHO, human-animal interface
http://promedmail.org/post/20160523.4239090
Avian influenza, human (44): China, H7N9, WHO
http://promedmail.org/post/20160518.4228384
Avian influenza, human (43): China, H5N6, mutations, WHO
http://promedmail.org/post/20160507.4205906
Avian influenza, human (34): China, H5N6, H7N9, WHO
http://promedmail.org/post/20160325.4118113
2015

Avian influenza, human (133): WHO assessment human-animal interface
http://promedmail.org/post/20151220.3881202
Avian influenza, human (115): human-animal interface, SA status
comments http://promedmail.org/post/20150718.3520025
Avian influenza, human (107): WHO assessment

http://promedmail.org/post/20150521.3376485]
.....sb/uba/ao/ml
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From: Jonna Mazet <jkmazet@ucdavis.edu>
To: Dennis Carroll <DCarroll@usaid.gov>;Eddy Rubin <erubin@metabiota.com>;Peter Daszak <daszak@ecohealthalliance.org>;Nathan Wolfe <nwolfe@metabiota.com>
Sent: 4/23/2017 8:17:57 PM
Subject: Fwd: An interesting read related to Ebola in West Africa

FYI,
J

---------- Forwarded message ----------
From: **Brian Bird** <bhbird@ucdavis.edu>
Date: Tue, Apr 18, 2017 at 1:12 PM
Subject: An interesting read related to Ebola in West Africa
To: David J Wolking <djwolking@ucdavis.edu>, Karen Saylors <ksaylors@metabiota.com>, Tracey Goldstein <tgoldstein@ucdavis.edu>, Simon Anthony <sja2127@cumc.columbia.edu>, Jon Epstein <epstein@ecohealthalliance.org>, Jonna Mazet <jkmazet@ucdavis.edu>, Leilani Francisco <francisco@ecohealthalliance.org>, Frantz Jean Louis <fjeanlouis@metabiota.com>

I came across this today. Just sharing for general interest.

Goes into cultural aspects around Ebola in Sierra Leone and past inequities and current ones through the eyes of 5 individuals. It might go off the deep-end in places, but overall rings true to my ears and what I saw on the ground.

Especially this quote: "Insai di war, yu kin si yu enimi; Ebola yu nor dae siam." (During the war, you could see

your enemy; Ebola can't be seen.)

HHR
Health and Human Rights Journal

Biosocial Approaches to the 2013-2016 Ebola Pandemic

Eugene T. Richardson, Mohamed Bailor Barrie, J. Daniel Kelly, Yusupha Dibba, Songor Koedoyoma, and Paul E. Farmer

Abstract

Despite more than 25 documented outbreaks of Ebola since 1976, our understanding of the disease is limited, in particular the social, political, ecological, and economic forces that promote (or limit) its spread. In the following study, we seek to provide new ways of understanding the 2013-2016 Ebola pandemic. We use the term, 'pandemic,' instead of 'epidemic,' so as not to elide the global forces that shape every localized outbreak of infectious disease. By situating life histories via a biosocial approach, the forces promoting or retarding Ebola transmission come into sharper focus. We conclude that biomedical and culturalist claims of causality have helped obscure the role of human rights failings (colonial legacies, structural adjustment, exploitative mining companies, enabled civil war, rural poverty, and the near absence of quality health care, to name but a few) in the genesis of the 2013-16 pandemic. From early 20th century smallpox and influenza outbreaks to 21st century Ebola, transnational relations of inequality continue to be embodied as viral disease in West Africa, resulting in the preventable deaths of hundreds of thousands of people.

"To tear treasure out of the bowels of the land was their desire, with no more moral purpose at the back of it than there is in burglars breaking into a safe. Who paid the expenses of the noble enterprise I don't know..."
Joseph Conrad, Heart of Darkness (1902)

Eugene T. Richardson, MD, is a PhD Candidate in the Department of Anthropology at Stanford University, Stanford, CA, USA; and a Research Scientist at Partners In Health, Freetown, Sierra Leone.

Mohamed Bailor Barrie, MBChB, is co-founder of Wellbody Alliance, Koidu, Sierra Leone; an MMSc Candidate in the Department of Global Health and Social Medicine at Harvard Medical School, Boston, MA, USA; and Strategic Adviser to Partners In Health, Freetown, Sierra Leone.

Daniel Kelly, MD, is co-founder of Wellbody Alliance, Koidu, Sierra Leone; and a Lecturer in the Division of Global Health Equity at Brigham and Women's Hospital, Boston, MA, USA.

Yusupha Dibba, MBChB, is Medical Director of Wellbody Alliance, Koidu, Sierra Leone.

Songor Koedoyoma, MBChB, is Chief of Staff of the Kono District Ebola Response Center (DERC), Koidu, Sierra Leone.

Paul E. Farmer, MD, PhD, is co-founder of Partners In Health, Boston, MA, USA; Kolokotrones University Professor of Global Health and Social Medicine at Harvard Medical School, Boston, MA, USA; and Chief of the Division of Global Health Equity at Brigham and Women's Hospital, Boston, MA, USA. He is Editor-in-Chief of *Health and Human Rights Journal.*

Please address correspondence to Eugene T. Richardson, MD, Department of Anthropology, Stanford University, 450 Serra Mall, Main Quadrangle, Building 50, Stanford, CA 94305-2034, USA. Email: etr@stanford.edu

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Introduction

The 2013-2016 Ebola pandemic is the largest and longest in recorded history, with more than 28,600 reported cases as of December 13, 2015.[1] The *proximal* determinants of the outbreak in West Africa have been well described: dysfunctional health services, a highly mobile and interconnected population, debilitated government institutions, burial practices that involve contact with contagious Ebola-infected corpses, and unsafe and poor quality provision of care to infected individuals.[2] More distal determinants, including slave-trading, exploitative colonialism, enabled civil war, resource extraction, and unethical pharmaceutical trials on African people, have received scant attention.[3] These centuries of extractive activities and rights violations have led to regional and global population movements which occur against a backdrop of rapid ecological change in the increasingly deforested regions of the three most affected countries—Sierra Leone, Liberia, and Guinea.

Despite more than 25 documented outbreaks since 1976—and, likely, uncounted others prior to the identification of the virus—our understanding of Ebola is limited, particularly concerning the social, political, ecological, and economic forces that promote its spread.[4] For the most part, analytic attempts have had the effect of removing health and illness from the social and political context in which they are produced.[5]

Biosocial analyses of disease burdens increasingly recognize the limitations of biomedical and epidemiological studies, which often ignore the economic and political factors resulting in poor health outcomes.[6] Biomedical culture's linear notion of progress can be contrasted with the interactive and systems orientation of more biosocial paradigms.[7] These paradigms provide a lens with which to view "the swirling political and economic relationships that dialectically produce levees and slums, soils and dams, tourism and hunger, energy and climate," health systems and pandemics.[8] Like studies of structural or symbolic violence, they can integrate "power into the understanding of disease dynamics."[9]

In the following study, we provide a broadly biosocial analysis of the 2013-16 Ebola pandemic, one that leads us "from the large-scale to the local and from the social to the molecular."[10] By situating life histories in a biosocial approach, the forces promoting Ebola transmission come into sharper focus.[11]

Brief background on Sierra Leone

Sierra Leone is a West African nation of 6.1 million people with a gross national income per capita of US$710 and a life expectancy at birth of 45 years.[12] Referred to as "the white man's grave," the country was established by Britain as a repatriation colony for former slaves in 1787.[13] It gained independence from the United Kingdom in 1961 after more than 150 years of colonial rule.

The country is divided into 14 districts and 149 chiefdoms, the latter traditionally ruled by Paramount Chiefs. Kono District (Figure 1) borders Guinea and is notable for diamond mining, thus attracting a significant amount of migrant labor. The district was also one of the major theaters of the Sierra Leone civil war (1991-2002), a conflict that resulted in more than 70,000 casualties and 2.6 million displaced

FIGURE 1. Sierra Leone and surrounds. Kono District in red. Source: Centers for Disease Control and Prevention

people, many of them across the borders with Guinea and Liberia.[14]

Materials and methods

PEF designed the study, which was conducted from December 2014 to March 2015. ETR and JDK first gathered systematic observations of triage, isolation, treatment, discharge, and burial while working as clinicians at the Koidu Government Hospital holding center for Ebola and in four Ebola Community Care Centers run by Partners In Health in Kono District, Sierra Leone. ETR compiled field notes nightly.

ETR and MBB then conducted open-ended, in-depth, semi-structured interviews with four Ebola survivors from Kono District. Survivors were recruited by contacting the director of the Kono Survivors' Group and requesting that she identify a miner, student, farmer, and health care worker who would be interested in being interviewed. Written informed consent was obtained and participants were reimbursed 50,000 Leones (US$10) for their participation. Interviews were conducted in Krio and audio-recorded. Members of the research team discussed findings and interpretations until consensus was reached on the dominant themes. Ethnographic data were then integrated with historical, political, economic, and ecological evidence to provide a biosocial account of the pandemic.

Ethics approval was not obtained, as the Stanford Institutional Review Board does not require protocol submission for research data from fewer than five participants. Names have been changed to protect confidentiality.

Results

1. Sahr (miner)

"Lei dem nor kam nia mi." *(Don't let them near me.)*

Sahr G. was born in Kono District in 1984, but spent most of his early years in the nearby district of Bombali. When his father died in 1994, he moved back to Kono to begin work as a child miner. In 1996, at 12 years old, he was captured during the Revolutionary United Front's (RUF) siege of Koidu (the capital of Kono District) and forced to join the fighting. The RUF's policy of capturing and enlisting child soldiers during the Sierra Leone Civil War is well documented, with one post-war survey suggesting that 88% of all RUF combatants were recruited via abduction.[14] Sahr was shot during a battle with government troops but survived. In 2002, he participated in the Disarmament, Demobilization and Reintegration (DDR) process, turned in his prized German submachine gun, and was given 300,000 Leones with which he planted a palm farm that has yet to turn an adequate profit.

Sahr resumed mining after the war, finding several large alluvial diamonds in the Sewa River. He invested his earnings in what turned out to be a Nigerian investment scam called "Wealth Builders." He had planned to migrate to Europe with his earnings, but instead was obliged to resume panning the region's rivers and streams.

In December 2014, Sahr's uncle became gravely ill with acute fever and gastrointestinal symptoms and was brought to Koidu Government Hospital (KGH). At triage, he was misdiagnosed as being Ebola-negative and sent home. Due to an Ebola "sensitization" campaign in the district, Sahr knew the risks associated with touching suspected Ebola cases but was confident in the hospital's judgment that his uncle was negative: "*A grip di pa; a fid am sup.*" (I held my uncle; I fed him soup.) Sahr cared for his uncle and started showing similar symptoms himself the day his uncle passed away. By then, he was suspicious enough that Ebola was the culprit to warn his family, "*Lei dem nor kam nia mi.*"

Sahr then hired a motorcycle, rode to KGH, was admitted, and tested positive for Ebola. He was sent to the Ebola treatment unit (ETU) in Kenema since Kono still lacked such a facility. The ambulance was packed with seven positive patients; two died en route. On arrival at the Kenema ETU, he panicked when he saw the red fences surrounding the suspect/confirmed zone: the rebels had attached red cloths to their weapons during live battles. Of the 14 patients sent to Kenema that day, only two survived. Sahr described it as the worst experience of his life, worse than any battle he had fought in

the civil war: "*Insai di war, yu kin si yu enimi; Ebola yu nor dae siam.*" (During the war, you could see your enemy; Ebola can't be seen.)

2. Aminata (student)

"Wetman no dae get Ebola bikos i dae tek meresin." *(White people can't get Ebola because they are protected by their medicines.)*

Aminata K. was born in Sandor Chiefdom, Kono District, in 1993. She spent her childhood going to school and helping her father farm his small plot. She and her family were forced to flee to Guinea during the third RUF campaign in 1998.[15] For over a week, they trekked by night and hid in the bush by day, eating bananas and rice their father brought, traveling toward the refugee camps in Guinea. Camp life across the border proved very difficult: her family was forced to sleep outside until her father gathered enough materials to build them a hut. Food was also scarce, and her father eked out an income by gathering firewood from local forests. After a year and a half in the camp, they returned to their village, which had been completely razed. Her father had hidden a drum of rice in the forest prior to their exodus, and it fed them until they could replant and harvest their farm.

When Aminata was 13, her mother died from an unknown illness. Her elder sister died in childbirth the same year.[16] Even before Ebola spread west across the country in 2014, Sierra Leone had the highest maternal mortality ratio in the world. The lifetime risk of maternal death in Sierra Leone is 1 in 21; by comparison, the same figure in Spain is 1 in 15,000.[17]

After Aminata's father remarried, conflict developed between Aminata and her stepmother, so her father moved Aminata to Koidu to finish her education. On account of schools closing during the war—when some 70% of educational institutions in Sierra Leone were destroyed—Aminata entered her final year of high school when she was 21.[18] Due to Ebola, all schools in Sierra Leone were closed from June 2014 to April 2015; a notable rise in teenage pregnancies ensued.[19] During this period, Aminata started a relationship with a 38-year-old pastor. They planned to wed, Aminata said, but she knew to stay away when 10 of the 15 people in his household came down with Ebola. The pastor was symptom-free during this catastrophe, but decided to jump in the back of the ambulance when his sick mother was being transferred to the ETU in Kenema, partly in order to get himself tested. After two negative Ebola tests, he went to a friend's house in Koidu to avoid quarantine in his home. Three days later he fell ill with symptoms typical of Ebola. Aminata decided to care for him, reassured by his negative results (and the accompanying document certifying them). Shortly thereafter, the pastor was admitted to the Kono ETU (which opened in January 2015) and tested positive for Ebola. Aminata was admitted with similar symptoms five days later, and tested positive, but was separated from the pastor by a double boundary of red fencing. When asked why Aminata didn't request to join her fiancé, she replied, "*Usai dem put mi, na dae a dae.*" (Where they put me, that's where I should be.)

Both Aminata and the pastor were discharged as survivors, and they followed the instructions of the discharge nurses not to engage in sexual relations. The pastor died from apparent liver failure 10 days after he was discharged. Like most Ebola survivors, he had received little in the way of immediate clinical follow-up (in spite of mounting evidence of near- and long-term sequelae). When he became jaundiced, they consulted an herbalist, believing that he had been cured of Ebola, and was now "*kontri sik.*" That is, his discharge certificate verified that he was a survivor of Ebola—a foreign illness or construct; the subsequent jaundice was thought to be a result of witchcraft. Since her own discharge, Aminata has developed post-Ebola uveitis, an increasingly recognized sequela.[20]

3. Tamba (farmer)

"Dem nor dae kam nia yu cos yu bin get Ebola. Dem say, 'wi no wan yu na wi ples agen.'" *(People don't come near you when you've had Ebola. They say, 'We don't want you near our place again.')*

Tamba L. was born in Kambia District (on the

border of Guinea) in 1949. His father was a 'master farmer,' producing more than 100 bushels of rice per year. When Tamba was 10 years old, he and his elder sister moved to Nimikoro Chiefdom in Kono District, and she trained him as a tailor. He married in 1971 and had five children. He began farming, producing about three bushels of rice per year. His father was well connected to the Paramount Chief in Kambia and was thus able to get farm support (for example, access to a tractor and fertilizer) via the patronage system, or 'clientelistic exchange.'[21] Tamba lamented that "big man" partnerships in Kono revolved around mining, and because of this, he was never able to enter the patrimonial system of politics there.

Like much of Kono's population, Tamba was displaced during the civil war and fled to Makeni, then Kambia, and finally to Freetown. When he returned to Kono after the war, he had to start over: "*A don los evri tin.*" (I lost everything.)

Ebola struck Tamba's village of Bumpe iatrogenically, and was then amplified through funeral practices. A well-respected taxi driver from Bumpe ferried a sick woman to KGH from the heavily afflicted village of Ndogboi. Around 10 days later, the driver suffered a stroke and was brought to KGH. At that time, the hospital was not taking admissions—the wards were being decontaminated—so the doctors in triage had two options for all presenting patients: 1) send them as Ebola suspects to the Kenema ETU, four hours away, or 2) send them closer to home to receive care in small health posts known as Peripheral Health Units. Hospital records indicate that the driver did not meet suspect criteria and was thus sent back to his village. He died several days later, and the District Ebola Response Center (DERC) was then called. The DERC sent a team to swab the body for Ebola polymerase chain reaction (PCR) testing; the body was left in the village pending the results. The results took three days to come back; this, combined with the driver's dismissal from the hospital, led many villagers to believe he did not die from Ebola. They held a funeral: "*Alman kam na di berrin.*"(Everyone came to the burial.) The Ebola swab came back positive, and the subsequent village outbreak killed more than 40 people, including Tamba's wife and their nursing child. Tamba tested positive shortly after, showing symptoms, and was quickly transported to the Kenema ETU with four friends, all of whom died.

When Tamba was asked about the reception he received in his village after discharge, he said: "*Dem nor dae kam nia yu cos yu bin get Ebola. Dem say, 'wi no wan yu na wi ples agen.'*" Soon after returning home, he was employed as a community health educator as part of the Partners In Health Survivors' Program. Tamba believes community sensitization campaigns are the key to controlling future outbreaks; he says, "*Patna Pa Welbodi don gi mi wok fo go tich di villej pipul dem. Naw wi dae it wan ples...en do evri tin in komon.*" (Partners In Health gave me work to educate the community. Now we eat in one place... we do everything together.)

4. Esther (nurse)

"Usai dem tai kaw, na dae i dae it." *(Where you tie the cow, that's where it eats.)*

Esther B. was born in Nimiyama Chiefdom, Kono District, in 1986. Her father died before she was born, and she grew up as an only child with her mother, a typist for the chiefdom. During the war, Esther and her mother sought refuge in Freetown, where she completed secondary schooling. In 2006, she returned to Kono to work as a volunteer nurse's aide at KGH. For seven years, she lived on handouts from staff nurses, and was finally invited to attend nursing school in 2012. This is a common path for young people interested in nursing in Sierra Leone. When asked why individuals would tolerate such hard work without steady pay, she replied, "*Usai demtai kaw, na dae i dae it.*" Figuratively speaking, she explained, this means that if one works as a nurse, this is how she should gain her livelihood; she should not be expected to beg outside the hospital (that is, cut the tie and go look elsewhere for food) but rather within it. Even 'fully employed' nurses are so underpaid that they are forced to charge patients for otherwise free drugs and services. Instead of recognizing nurses' salaries

as insufficient to live on, the government (and even more non-Sierra Leonean commentators in the aid industry) terms this behavior "corruption." (Figure 2)

In October 2014, Esther responded to a job request from the district to work as a nurse at the Ebola holding center at KGH. She was promised USD 100 per week (the standard Sierra Leone Ebola hazard pay) for 10 weeks of work. To date, she has received USD 20. On a single day in December, we witnessed conditions at their worst: over 20 corpses lying in the ward, hygienists spraying moribund patients in the face with bleach to see if they were alive or not, hundreds of pounds of contaminated personal protective equipment lying about, and heroic nurses doing their best despite the deplorable conditions.

As elsewhere, such conditions led to nosocomial infections. By January 2015, all nine of the nurses who worked in the center had contracted Ebola; seven died. After recovering from her illness, Partners In Health hired Esther as a nurse at one of their Community Care Centers. These centers are part of a decentralizing strategy, bringing small Ebola treatment units to relatively underserved communities—and employing survivors.

Discussion

Biosocial analysis represents a fluid space between the disciplines of economics, anthropology, political science, history, ecology, epidemiology, and physiology.[22] In the case of the 2013-16 Ebola pandemic, it allows us to interrogate claims of causality while insisting on the importance of the right to health. We use the term, 'pandemic,' and avoid 'epidemic,' so as not to elide the global forces that shape *every* localized outbreak of infectious disease.

Political economy

The survivors' experiences outlined above offer evidence of an abysmal public health infrastructure in Sierra Leone, as well as a near absence of clinical care. It thus seems germane to ask why health services—and government institutions in general—are so impoverished.

As Englebert has shown, the arbitrariness of a country's boundaries in Africa correlate with poor development outcomes.[23] The boundaries of Sierra Leone have had little to do with cohesive self-determination, but rather were determined by the interests of colonial powers.[24] In short,

FIGURE 2. Billboard at Koidu Government Hospital. Photo credit: ET Richardson

the Republic of Sierra Leone inherited a flawed, exploitative polity from colonial rulers.[25]

The heterogeneity of identities lassoed within such contrived boundaries help set the climate for patrimonial rule.[26] Patrimonialism, as highlighted in Tamba's narrative, is a "style of governance where politicians control power through a system of personal relationships where policies/favors are distributed in exchange for political support."[27] It is has been the *de facto* form of rule in Sierra Leone since independence was declared, and has been potentiated by minerals as a ready source of patronage.[28] Diamonds, for example, have been central to transnational corporate profiteering and underdevelopment in Sierra Leone.[29] They also triggered and sustained horrific brutality in the Sierra Leone civil war (1991-2002) and have come to be seen as a 'resource curse.'[30] What would it take to help remedy the situation in Kono, where billions of dollars have been extracted from the ground (Figure 3), yet roads, hospitals, and schools are absent or woefully underfunded? For example, while KGH lacked x-ray capability—making care for conditions ranging from tuberculosis to fractures suboptimal at best—the nearby mining company had a perfectly functional, if rarely used, x-ray machine.[31]

Patrimonialism as a system of rule is said to be counterproductive to economic development, representative government, and accountable institutions. As a violation of the right to health, resources that could be devoted to public works for the poor are instead funneled to elite coffers—hence the "public health desert" found in today's Sierra Leone.[32] The resulting dysfunctional health facilities then amplify transmission in infectious disease outbreaks: as of December 13, 2015, Sierra Leone reported 14,122 cases of Ebola.[33] Indeed, Esther's tally of nine out of nine nurses infected at the KGH holding center demonstrates the parlous risk dysfunctional health facilities pose.

As an example of the lack of checks and balances associated with patrimonial governance, and because of weak public institutions, Sierra Leone did not have an analytic platform capable of accounting for all the money allocated to fight Ebola, most of which went to international NGOs and contractors. A 2015 report by national auditors found that almost a third of these funds were unaccounted for.[34] When we asked our national staff where the money went, many replied, "*Dem don it di moni.*" (The government elites ate [stole] the money.) We witnessed the patrimonial system at work as foreign aid was channeled through the National Ebola Response Center, then on to District Ebola Response Centers, some headed by Paramount Chiefs. Resources (for example, vehicles) were meted out according to informal arrangements, rather than actual UN- and donor-written allocations. Esther's unresolved hazard pay was another casualty of such a system.

But government institutions are not solely to blame. The paradigm of infectious disease exceptionalism, whereby international aid is funneled into intensive responses for exceptional conditions (HIV, for example), reorients the donor gaze from strengthening health systems in general, to mopping up preventable pandemics.[35] The aid industry thus serves as an "anti-politics machine," which effectively casts problems of outbreak containment in apolitical, ahistorical, techno-managerial terms, while dis-

FIGURE 3. Diamond (kimberlite) mine as viewed from UN helicopter. Photo credit: ET Richardson

guising the underlying political and economic causes.[36] Another way of saying this, is that purposeful underdevelopment in West Africa and trans-hemispheric relations of inequality are the real pathologies—not Ebola.

This leads us to question: Is current Ebola aid from the World Bank, the United Kingdom Department for International Development, and the United States Agency for International Development enough to redress the path-dependent influence of colonial institutions that have facilitated the current pandemic?[37] Is this aid a form of reparations disguised as altruism? Will this outpouring of Ebola aid lead to sustainable investments and health-systems strengthening in Sierra Leone? The huge reductions in aid (now that West Africa has been declared 'Ebola free') suggest otherwise. As Sahr observed, "*Wei di ren dae kam, yuba sae i go bil os; wei san komot, i foget.*" (When it's raining, the vulture says he'll build a house; when the sun comes out, he forgets.)

Human and peoples' rights

The 1981 African Charter on Human and Peoples' Rights (also known as the Banjul Charter) is a rather novel instrument in that it not only includes rights for individuals but also for people.[38] The promulgation of the right to an environment conducive to satisfactory collective development (Article 24) provides a moral language for biosocial analyses, in that it transcends the liberal focus on 'bounded' individuals and pays special recognition to human interconnectedness.[39] Esther's narrative demonstrates that health care workers (HCWs) have risks that are structured by their group identity. When the term "caregiver" is extended to include people like Sahr, Aminata, and Esther—all of whom provided care for infected people—we see that Ebola is fundamentally a disease of people who care about others, but lack the staff, stuff, space, and systems to do so safely.

Relational human rights in West Africa are not solely promoted by a mere exposé of how the fruits of scientific advances are stockpiled for some and denied to others.[40] Rather, there must be a praxis component. It forces us to ask: Will the rebuilding of the country's economy and health infrastructure occur in pragmatic solidarity with the people of Sierra Leone—as in the case of Tamba? Or will Western aid continue to manage "inequality with the latest tools from economists and technocrats"?[41]

Using the Banjul Charter as a guide, we witnessed relational human rights violations throughout the Ebola outbreak: in WHO's initial mishandling of the public health threat; in dysfunctional health systems; in quarantined villages that lacked adequate food supplies; in extractive biomedical and social science research programs lacking clinical delivery platforms; and in the limited use of intravenous resuscitation—the only hope to save those with significant loss of fluids and electrolytes—throughout the brunt of the outbreak. Even Médecins Sans Frontières (MSF), recipient of the Lasker-Bloomberg Public Service Award in recognition of their work in the Ebola-affected countries, remarked, "the prolonged reluctance of placing IV-lines to severely dehydrated patients will remain a symbol of the inadequate clinical care during MSF's Ebola intervention in West Africa."[42] All of the NGOs on the ground had the responsibility to provide good supportive care to those suffering from Ebola.

Ecology

Several authors have posited ecological forces in sparking outbreaks of Ebola. Bausch and Schwarz describe a three-tiered cascade that results in sustained outbreaks: 1) poverty drives individuals to encroach deeper into forests, making zoonotic transmission more probable; 2) infections are then amplified by a dysfunctional health system; and 3) containment is hampered by poorly resourced governments.[43] Such a thesis is plausible and far better buttressed by evidence than are culturalist claims, but more could be said. When Tamba was presented with this line of reasoning, he remarked, "*Wi don dae go na bush fo yia, pan di wa...en di ospital dem bin bad. So wai naw?*" (We've been going deep in the forest for ages, especially during the war...and the hospitals have

always been bad. So why now?) While Tamba is not aware of recent evidence that demonstrates circulating Ebola prior to this outbreak, he is prescient to ask, "Why now?"[44]

Another important question is by what mechanisms has international agribusiness shaped and taken advantage of the 2013-2016 Ebola pandemic. Wallace and colleagues posit that capital-intense industrial palm oil farms have increased human interaction with frugivorous bats (*Pteropodidae*), a putative Ebola reservoir, and may explain the association of outbreak peaks at the start of the dry season, when oil palm picking is at its height.[45] Alarmingly, a recent report by Global Witness documents the way Golden Veroleum Liberia (a subsidiary of Golden Agri-Resources, a palm oil plantation company based in Indonesia) has taken advantage of pandemonium and the scaling back of advocacy by environmental NGOs during the pandemic to double its landholdings for palm oil plantations.[46] The dependence on subsistence farming, which Aminata describes, should stimulate interrogation of trade deals that often undermine food security.

Climate change may be another ecological mechanism that contributes to Ebola outbreaks. Alexander and colleagues suggest that increasing climate variability can alter fruiting patterns in West African flora, potentially concentrating reservoir and susceptible host species in areas of increased foraging opportunity. This can lead to increased Ebola transmission in wildlife, which may result in a higher probability of spillover to humans. The authors thus conclude, "research into Ebola reservoir and transmission dynamics will be essential to refining surveillance approaches."[47]

Like the environment itself, ecological claims are fragile and uncertain. Fairhead and Leach provide a compelling challenge to straightforward models of ecological change, arguing that deforestation has consistently been exaggerated in this region which has long been densely populated and farmed and in which many of the forests are actually anthropogenic in origin.[48]

Do inequitable trade agreements facilitate the ecological conditions conducive to pandemic disease? Is it possible to tie unsustainable consumption and carbon emissions in industrialized countries to increased outbreaks of viral hemorrhagic fevers? Do anthropogenic forests further facilitate spillover? We agree with other authors that ecological analysis and policy need to be reframed towards addressing the problems of socially vulnerable people, like Tamba, Aminata, and Sahr.[49]

"Culture" and claims of causality

The term "culture" is perhaps the most vexed in anthropology, and in the social sciences in general; it admits to multiple meanings in discussions of serious illness, where it serves as a black box with extraordinary (and often contradictory) explanatory power. It is certain that the cultural traditions of the people most affected by Ebola, and those charged with responding to it, have shaped the current pandemic in significant ways; these traditions have included ways of understanding illness, of seeking care in the absence of a functioning health system, and in funerary practice in the absence of almost anything in the way of assistance for family members. But much more can be said about the influence of rapidly changing social institutions and ecological conditions on what are loosely (and inaccurately) termed "traditional beliefs." One classic example is the fetishization of bushmeat and bats. The inordinate amount of attention focused on bushmeat and bats in radio programs, pamphlets, billboards (see Figure 4), and other media culturalizes and de-historicizes the political and economic forces that have promoted the pandemic. It should be remembered that only one of the more than 28,000 reported cases in West Africa is thought to have come from bushmeat or bats.[50]

Concerning bushmeat, Rizkalla and colleagues argue, "Despite efforts to change the eating habits of African villagers, many believe occult forces are behind Ebola. They do not understand that they could limit their exposure by avoiding dead or sick animals."[51] Many of the patient histories we took while working at the Community Care Centers were at odds with the

view that intractable behaviors amplify transmission. These histories are corroborated by the in-depth interviews with Sahr, Aminanta, and Tamba, each of whom indicated they were aware of no-touch policies, but were dissuaded by the ambiguous—in their view—discharge certificates that signaled their contacts were Ebola-free. They also stepped in where professional caregivers were unavailable, beleaguered, or dead.

The cultures of distinctly transnational institutions have also shaped the Ebola pandemic. As we observed at inter-agency meetings, providers of foreign aid clamored to publish the number of ETUs built, patients triaged, lab specimens processed, calls made to 117 (the national Ebola alert number), and so on, while often failing to ask critical questions such as: Is chronic poverty obscured by international interventions that fetishize epidemiological statistics and 'performance' indicators?[52] Such statistics, and their exaggerated precision, evoke the power of scientific claims and make it "difficult to distinguish an emergency [outbreak] from chronic poverty."[53] In addition, with the input of anthropologists, these statistics tend to reify culture "as an ensemble of measurable factors with deterministic power over specific aspects of illness."[54] In other words, the emphasis on 'deliverables' in the emergency setting obfuscates the relationship between human rights failings and outbreaks of infectious disease.

A final example is biomedical culture's focus on Ebola as a freestanding biological entity, which disguises its existence as a social phenomenon.[55] As an illustration, we observed that Ebola 'survivors'—that is, those who are symptomatic, test positive, and survive—were sometimes given benefits in the form of food, clothing, and employment, even if they were the only ones in their families affected. Suspect cases who were discharged negative from an ETU were given nothing, even if all their family members perished on account of the disease. In short, the biomedical notion of individual symptomatic viremia determined whether or not "an individual" received international aid, which may subvert Sierra Leonean collectivist psychologies.[56] In recognition of these relational rights, some NGOs provide social support for Ebola-affected persons and their networks, whether an individual tests positive or not.

Points of departure

From early 20th century smallpox and influenza outbreaks to 21st century Ebola, transnational relations of inequality continue to be embodied as viral disease in West Africa, resulting in the preventable deaths of hundreds of thousands of individuals.[57] Then and now, biomedical and culturalist claims of causality help obscure the role of human rights failings in the genesis of infectious disease outbreaks. We submit that an analytic framework based on radical relationality, including webs of power relations at the political, economic, ecological, and cultural levels, is necessary to provide an adequate account of the re-emergence of Ebola in West Africa.[58] These webs stretch back in time and across the region and link it to distant continents, as a more com-

FIGURE 4. Bushmeat billboard, Liberia. Photo credit: P. E. Farmer.

prehensive account of the current pandemic will one day reveal—and as Sahr's, Aminata's, Tamba's, and Esther's accounts already suggest. An historically grounded, biosocial approach can foster a change towards a more reflexive understanding of outbreak responses in general, one that provides a corrective lens for biomedical tunnel vision.

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From: Andrew Clements <aclements@usaid.gov>
To: Dennis Carroll <dcarroll@usaid.gov>;tom.hughes@ecohealthalliance.org <tom.hughes@ecohealthalliance.org>;Timothy Meinke (HANOI/OH) <tmeinke@usaid.gov>;Daniel Schar (RDMA/OPH) <dSchar@usaid.gov>;Bambang Heryanto <bheryanto@usaid.gov>;William Karesh <Karesh@ecohealthalliance.org>;daszak@ecohealthalliance.org <daszak@ecohealthalliance.org>;Jonna Mazet <jkmazet@ucdavis.edu>;Alisa Pereira <apereira@usaid.gov>;djwolking@ucdavis.edu <djwolking@ucdavis.edu>;sgillette@usaid.gov <sgillette@usaid.gov>
Sent: 4/25/2017 12:56:30 PM
Subject: NYTimes: A Refuge for Orangutans, and a Quandary for Environmentalists

FYI
>
> https://www.nytimes.com/2017/04/25/world/asia/indonesia-borneo-orangutans-palm-oil.html?smid=nytcore-ipad-share&smprod=nytcore-ipad
>
> Some worry that a palm oil company's gift to endangered Borneo orangutans distracts from a big threat to the species: deforestation by such companies.
>

From: "Jonna Mazet" <jonna.mazet@gmail.com>
To: <Karesh@ecohealthalliance.org>
Cc: [redacted]
Subject: Reminder: Invitation to participate in virus risk ranking assessment
Sent: Wed, 3 May 2017 14:24:18 -0700
RiskRankingPartcipantWorksheet.xlsx

Dear Dr. William Karesh,

We hope that you previously received our email soliciting your expert opinion and requesting your participation in a short multidisciplinary process to assess spillover risk from newly detected viruses. As an expert in the field of infectious diseases, your contribution to this exercise would be highly valued and appreciated. If possible, please take a moment of your time to review the information below, and complete the attached worksheet. We anticipate the time allocation to this exercise will be **10** to **20 minutes**.

As you may have heard, the USAID-supported PREDICT project (www.predict.global) has identified short sequences from nearly 1000 unique viral taxonomic units (by consensus PCR followed by sanger sequencing) from viral families known to have members that cause zoonotic diseases. These viruses have been detected in samples collected from animals in more than 20 countries in tropical regions considered to be hotspots for emerging zoonotic disease risk.

As a globally renowned scientist in the field of infectious diseases, we would like to incorporate your expert opinion into an evaluation of the relative impact that select host, environmental, and viral factors contribute to the risk of a new human viral spillover or epidemic event that might originate from novel or known viruses of animal origin. At this point, we are primarily interested in how much each parameter contributes to the overall risk of such an event occurring. The levels of severity within each of the parameters will be evaluated through a different process.

The expert opinion you provide will be combined with that of other top experts in the field and is intended to contribute to a risk ranking module that will be distributed to and evaluated by the scientific community both through the peer-reviewed publication process and via an interactive web application. All contributions to this exercise are voluntary, and identifying information will not be published or be otherwise made available unless you let us know that it is acceptable/desirable to acknowledge you. We are only soliciting opinions from a select group of professionals with relevant expertise; therefore, we ask that the attached worksheet remain confidential and not to be shared with others.

Instructions:

1. Please open and save the worksheet with your initials in the title (i.e. RiskRankingPartipantWorksheet_ZG.xlsx)
2. Complete the 'Demographic Information' at the top of the spreadsheet
3. Answer all categories for 'CONTRIBUTION TO THE RISK OF A NEW HUMAN VIRAL SPILLOVER OR EPIDEMIC EVENT OF ANIMAL-ORIGIN' and 'LEVEL OF EXPERTISE' using provided dropdown options
4. Please return your completed worksheet ASAP to [redacted] original deadline **April 28th 2017** extended for your participation to May 12 or by arrangement if this date is impossible and you would still like to contribute**.**

We sincerely hope that we can count on your important involvement in the process and that you will accept our gratitude for your time and contribution to scientific collaboration.

Sincerely,

Prof. Jonna Mazet

Global Director, PREDICT USAID
Professor of Disease Ecology and Epidemiology
One Health Institute
School of Veterinary Medicine
University of California Davis

[redacted]

Project Scientist, PREDICT USAID
Postdoctoral Researcher in Disease Ecology
One Health Institute
School of Veterinary Medicine
University of California Davis

1089 Veterinary Medicine Drive
Davis, CA 95616, USA
jkmazet@ucdavis.edu

1089 Veterinary Medicine Drive
Davis, CA 95616, USA
REDACTED

Produced in Native Format

From: Andrew Clements <aclements@usaid.gov>
To: Tracey Goldstein <tgoldstein@ucdavis.edu>
CC: Jonna Mazet <jkmazet@ucdavis.edu>;David Wolking <djwolking@ucdavis.edu>;Alisa Pereira <apereira@usaid.gov>
Sent: 5/10/2017 12:49:15 AM
Subject: Re: SL update

Nice! Thanks.

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On May 9, 2017, at 11:18 PM, Tracey Goldstein <tgoldstein@ucdavis.edu> wrote:

Hi Andrew,

I have been in touch with the CDC today and the MTA paperwork is moving forward so hopefully we will have permits in place to ship those samples soon.

Best, Tracey

On Tue, May 9, 2017 at 10:07 AM, Andrew Clements <aclements@usaid.gov> wrote:
Thanks, Tracey. I have fingers and toes crossed that permission will be granted to ship and later publish the results before the next election. :)

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On May 9, 2017, at 5:53 PM, Tracey Goldstein <tgoldstein@ucdavis.edu> wrote:

Hi Andrew,

We got a similar report from Aiah this week.

We are working on the paperwork to ship the samples to CDC - an MTA is needed and is currently being reviewed from by the office in Atlanta. We will ship as soon as we have that in place. We are also finishing up a few things in the lab and working on a draft of a publication with the information we have to date. We are also working on a plan for community engagement once we are ready to move forward.

Will keep you posted.
Best, Tracey

On Tue, May 9, 2017 at 2:13 AM, Andrew Clements <aclements@usaid.gov> wrote:
Hi all,

Kendra provided an update based on her recent trip.

Apparently, James Bangura drove from Sierra Leone to Guinea with the Chief Medical Officer and spent a lot

of time with the Minister of Health. He recommended that they approve sending the live virus to CDC to see if human cells can be infected and explained why this is important. He said they seemed to agree and asked James and Aiah to follow-up with them this week when they are all back in Sierra Leone.

They said nothing has changed with the President-- he does not want the info made public at this time. The USG position appears to now be that we should make no further requests to MOH or the President to release the surveillance findings unless we have new information.

So it appears that the way forward is to work on getting the samples/virus sent to CDC. Hopefully, the path is cleared for you to do this. If you encounter resistance, please let me know so we can discuss.

Thanks!

Andrew

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

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(530) 752-0412
(530) 752-3318
tgoldstein@ucdavis.edu

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(530) 752-0412
(530) 752-3318
tgoldstein@ucdavis.edu

Sent: Tue, 23 May 2017 09:48:15 -0700
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update
From: Jonna Mazet <jkmazet@ucdavis.edu>
To: Andrew Clements <aclements@usaid.gov>

I totally get it,
J

On Tue, May 23, 2017 at 9:10 AM, Andrew Clements <aclements@usaid.gov> wrote:

> Thanks for your understanding. Everybody has the best of intentions, but sometimes they get carried away trying to be super diligent.

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On May 23, 2017, at 4:56 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

> > Thanks very much, Andrew -- I think it's okay with just your message.
> > We'll see if we get more inquiries and go from there.
> > Appreciate your support,
> > Jonna
>
> On Tue, May 23, 2017 at 12:15 AM, Andrew Clements <aclements@usaid.gov> wrote:
>
> > Hi Jonna,
>
> Sorry about that. It's not uncommon for this to happen during outbreaks because more people are involved and sometimes they start reaching out directly to projects because of the real or perceived urgency with gathering information.
>
> See below for a note I sent back to Sarah and others. If you think it would be helpful, I can ask them to send all questions through me and I can filter out the unnecessary ones.

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

Begin forwarded message:

> **From:** Andrew Clements <aclements@usaid.gov>
> **Date:** May 23, 2017 at 9:10:39 AM GMT+2
> **To:** Sarah Paige <spaige@usaid.gov>, Angela Wang <awang@usaid.gov>
> **Cc:** PREDICTMGT <predictmgt@usaid.gov>
> **Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update**

I know there is a desire to get all the latest information available for outbreaks and that there are always a lot of questions, but for the sake of not bogging down our partners, let's try to keep our questions focused on activities related to how our partners are contributing. The partners efforts with the outbreaks (sometimes many at the same time) are in addition to their daily work in up to 30 countries so they only have a finite amount of time available to get work done.

Thanks.

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On May 23, 2017, at 2:28 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Thanks for your inquiry -- once again, this effort is not a Predict activity, so we don't have the details -- information from the meetings is provided for your notification, but we don't necessarily have the details that would be available from your in-country contacts. We will clarify in further updates that certain sections are informational items. If our personnel have any additional information, I will send on to you.

Have a good night,
Jonna

On Mon, May 22, 2017 at 2:01 PM, Sarah Paige <spaige@usaid.gov> wrote:

Thank you for the update, Jonna.
I have a question regarding this line from the report about the use of the Ebola vaccine. Does this mean that efforts are underway to clear the protocol through the regulatory authority and it will, for sure, be deployed?
Thank you!

- *The Government has approved the use of the Ebola vaccine in DRC during this Ebola outbreak.*
- *The Protocol of vaccination was submitted to Ethical Committee at KSPH for approval as a clinical trial.*
- *Several scenarios were proposed and will be discussed before starting the vaccination.*

Sarah Paige, PhD, MPH
Senior Infectious Disease Advisor
USAID Africa Bureau/Health Division
Desk: +1-202-712-1814
Mobile: REDACTED
E-mail: spaige@usaid.gov

On Mon, May 22, 2017 at 3:57 PM, Sarah Paige <spaige@usaid.gov> wrote:

Thanks All
I've also connected with our TB folks at HQ. I will share any further relevant info.

Best
Sarah

Sarah Paige, PhD, MPH
Senior Infectious Disease Advisor
USAID Africa Bureau/Health Division
Desk: +1-202-712-1814
Mobile: [redacted]
E-mail: spaige@usaid.gov

On Mon, May 22, 2017 at 1:41 PM, Angela Wang <awang@usaid.gov> wrote:

> Thanks! I will follow up to see if WHO has heard anything, since they are coordinating any supply requests and logistics around that.

On Mon, May 22, 2017 at 1:14 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

> FYI,
> Jonna

---------- Forwarded message ----------
From: **Karen Saylors** <ksaylors@metabiota.com>
Date: Mon, May 22, 2017 at 8:52 AM
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update
To: Tracey Goldstein <tgoldstein@ucdavis.edu>, Jonna Mazet <jkmazet@ucdavis.edu>
Cc: Brian Bird <bhbird@ucdavis.edu>, Damien Joly <djoly@metabiota.com>, Eddy Rubin <erubin@metabiota.com>, Maria Makuwa <mmakuwa@metabiota.com>, PREDICT-outbreak <predict-outbreak@ucdavis.edu>, Prime Mulembakani <pmulembakani@metabiota.com>, James Ayukekbong <jayukekbong@metabiota.com>

Hi Tracey and Jonna,
I just got off the phone with Prime and want to clarify a few things:
The idea of doing a joint sample collection trip with FAO was mentioned verbally at the Ebola coordination meeting but this has not been requested formally, in a written note from the Ministry, so currently, we are concentrating only on PREDICT supporting INRB in testing outbreak samples with PREDICT panels.

Regarding the thermometers: this is a question of logistical coordination for getting clinical supplies and consumables to the field, which is not PREDICT's domain. There are plenty of thermometers available in Kinshasa but the logistics arm of the response effort has had some challenges getting those to the outbreak site.

So Tracey, we are not yet collecting samples or storing them, but will certainly be attentive to cold chain if that effort is requested by the MoH.

Thanks,
Karen

From: [redacted] on behalf of Tracey Goldstein <tgoldstein@ucdavis.edu>
Date: Monday, May 22, 2017 at 8:35 AM
To: James Ayukekbong <jayukekbong@metabiota.com>, Jonna Mazet <jkmazet@ucdavis.edu>
Cc: Brian Bird <bhbird@ucdavis.edu>, Damien Joly <djoly@metabiota.com>, Eddy Rubin <erubin@metabiota.com>, Karen Saylors <ksaylors@metabiota.com>, Maria Makuwa <mmakuwa@metabiota.com>, PREDICT-outbreak <predict-

outbreak@ucdavis.edu>, Prime Mulembakani <pmulembakani@metabiota.com>
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update

Hi James,
Thank you for the update. Can you tell us a bit about how the samples are being collected and stored? Any details on the media they are using and cold chain would be helpful.
Best Tracey
On Sun, May 21, 2017 at 1:30 PM Jonna Mazet <jkmazet@ucdavis.edu> wrote:

> Thanks, James,
> Jonna
>
> On Sun, May 21, 2017 at 7:37 AM, James Ayukekbong <jayukekbong@metabiota.com> wrote:
>
> Dear all,
>
> Find attached the updated PREDICT Outbreak Rapid Report form regarding the current Ebola outbreak in DRC.
>
> We are told the Minister of health would sign an official request for PREDICT to perform the following;
>
> - To conduct a joined ecological research with FAO to look for Ebola virus among wild and domestic animals in Likati.
>
> - To test all samples (including negatives) from these outbreak with the PREDICT panel.
>
> Kind regards,
>
> **J.A Ayukekbong, PhD**
> Regional Coordinator /Central Africa
> USAID PREDICT | Metabiota
> Email: jayukekbong@metabiota.com
> Mobile: +1 250-797-7755
> Website: www.metabiota.com
> Skype: ayukekbong.ayukepi

--
Angela Wang, MSPH
Public Health Advisor
Emerging Threats Division, Office of Infectious Disease
USAID/Washington, Bureau for Global Health
Phone: 202-712-1070 (O) | [redacted]
Email: awang@usaid.gov

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From:	Jonna Mazet <jkmazet@ucdavis.edu>
To:	PREDICTMGT <predictmgt@usaid.gov>;Angela Wang <awang@usaid.gov>;Sarah Paige <spaige@usaid.gov>
CC:	PREDICT-outbreak <predict-outbreak@ucdavis.edu>
Sent:	5/24/2017 5:11:24 PM
Subject:	Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update

Hello,
Today's update attached. I observe in the meeting notes that there will be 4 aliquots of each sample tested and/or stored by the listed labs. Predict is not listed. I inquired with our team, and they responded that we have not yet received a letter officially requesting our testing. It is likely that if/when that is received, we will test the sample going to INRB, where our lab is located, but that is not yet confirmed. I also asked our team to confirm how the samples that we might be asked to test will be transported (media, cold chain, etc.) and stored.
Hopefully, we'll be able to provide clarity on that in the future,
Jonna

On Tue, May 23, 2017 at 9:56 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Current update attached.
Have a nice day,
Jonna

On Sun, May 21, 2017 at 1:38 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Please see below and attached regarding what has been requested of Predict and what we believe we can offer. Note that we are concerned about both cold chain and the media into which samples are being collected in the field. We can test the samples, but there will likely be a reduction in sample quality that may impact analysis.
We will keep you posted, but please let us know if you have questions that we should pass to the in-country team.
Have a nice day,
Jonna

---------- Forwarded message ----------
From: **James Ayukekbong** <jayukekbong@metabiota.com>
Date: Sun, May 21, 2017 at 7:37 AM
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update
To: Jonna Mazet <jkmazet@ucdavis.edu>, Maria Makuwa <mmakuwa@metabiota.com>
Cc: Prime Mulembakani <pmulembakani@metabiota.com>, PREDICT-outbreak <predict-outbreak@ucdavis.edu>, Brian Bird <bhbird@ucdavis.edu>, Eddy Rubin <erubin@metabiota.com>, Karen Saylors <ksaylors@metabiota.com>, Damien Joly <djoly@metabiota.com>

Dear all,

Find attached the updated PREDICT Outbreak Rapid Report form regarding the current Ebola outbreak in DRC.

We are told the Minister of health would sign an official request for PREDICT to perform the following;

- To conduct a joined ecological research with FAO to look for Ebola virus among wild and domestic animals in Likati.

- To test all samples (including negatives) from these outbreak with the PREDICT panel.

Kind regards,

J.A Ayukekbong, PhD
Regional Coordinator /Central Africa
USAID PREDICT | Metabiota
Email: jayukekbong@metabiota.com
Mobile: +1 250-797-7755
Website: www.metabiota.com
Skype: ayukekbong.ayukepi

PREDICT Outbreak or Health Event Rapid Report

Today's Date: *May 23rd, 2017*
Working Title of Investigation: *Outbreak of Ebola Virus Disease in the Bas-Uele province, DR Congo*
Cumulative day of the outbreak investigation: 14

Please describe the disease signs and symptoms and species affected (humans, domesticated animals, wildlife:

On 8 May 2017, an alert of 9 suspected cases of Human Viral Hemorrhagic Fever and 2 deaths in the Likati Health Zone, Bas-Uele Province was received from the Provincial Health Officer. Symptoms were fever, bloody vomiting, diarrhea, and bleeding from the nose.

Location	
Country:	*Democratic Republic of Congo*
District:	*Province of Bas-Uele, Health zone of Likati, north-west of Buta*
Village/Town:	*Village in the Nambwa health area, Territory of Aketi*
GPS Coordinates (if known):	
Date that first case(s) of illness occurred (if known or estimate):	*April 22nd, 2017*
Date that PREDICT was first notified of outbreak:	*On May 10th, 2017 the PREDICT CC was informed by the INRB staff working in the virology lab that they were notified of suspected cases of VHF in the Likati Health Zone and that samples were expected to arrive for confirmatory testing anytime.* *On May 11th, 2017 the PREDICT CC was informed that the samples arrived at INRB in early afternoon and are being tested for Ebola. The same day the PREDICT CC was informed by the EPT2 focal point at the mission who talked on the phone with the Bas-Uele provincial health officer about more details on this alert: 9 cases and 2 deaths.*

Key Information	**Description of Findings/Actions/Outcomes**			
How many affected individuals?		**Suspected:**	**Confirmed:**	**Deaths:**
	Humans	48	2	4
	Domestic Animals			
	Wild Animals			
How was outbreak first noticed?	*During 16th week, a 45 year old man (case 1), fisher and farmer, became sick with fever, then bloody vomiting, bloody stools and nosebleed in the fisher camp along the river Likati,*			

	*in the Nambwa health area. He was brought to a traditional healer and then transported by moto with 2 relatives, case 2 (moto driver) and case 3 (his brother) to the Likati general hospital about 45 km away. B*ut he died on the road. Then case 3 decided to return to their village with the corpse. He was buried in the Kapayi village, Nambwa health area. On 25th April, case 2 and 3 developed the disease with same symptoms. Case 2 died the same day, and case 3 recovered. From these 3 persons, 6 other close contacts were infected. Among them, a young boy who attended the burial of case 1 died on 11th May. The provincial health office has sent a team to the site to investigate and information is expected when they return as the area has no cell phone coverage.
Where was the first reported case? What is/was the extent of geographic spread? Include comments on the apparent speed of spread.	*For now the disease is located within four health centers: Nambwa (12 cases, 2 deaths), Muma (3 cases, 1 death), Ngayi (4 cases, 0 death) and Azande (1 case, 0 death), in the Likati Health Zone, Territory of Aketi in the Bas-Uele province, where the first reported case was treated at the health center. No case is reported outside this area.*
Has the country requested support from PREDICT (include date of request)?	*Yes, the INRB General Director asked PREDICT to retest the 5 samples that were received from the field using PREDICT protocols;*
If so, which government agency requested PREDICT support?	The Ministry of Health through the INRB which is the national Public Health Laboratory
When was PREDICT response initiated (date)?	Saturday, 13th May, 2017
Are other EPT partners involved in the response (which ones and how)?	*None for now*
What type of assistance did PREDICT initially provide? Which PREDICT personnel were involved?	Testing of 5 samples from the field using PREDICT protocols and primers for Filoviruses, by the PREDICT lab manager and lab technician
When was the first official acknowledgement of the outbreak (by which government agency or other reputable body and date)? When was a response initiated and by whom? Which agencies were involved? Who was in charge of the national response?	On May 9th, 2017, the Bas-Uele provincial office informed the MoH direction of disease surveillance of the alert. A team from Buta, the provincial health office was sent to the site to investigate. A team from the MoH direction of disease control, INRB, Hygiene and the Ministry of information travelled on Saturday morning to the field. They reached Likati (health zone office) on Sunday night at 10.00 PM. On Monday morning they had a meeting with the health zone staff and sent a first report to the national coordination committee via the Ministry of Health

Was the cause of the outbreak confirmed by a laboratory? If so, give details of the initial confirmation (cause, species, specimen types tested and dates of testing if known). *Note: Daily updates for ongoing laboratory testing should be entered in the Daily Activities/Timeline table below.*	Yes, the INRB virology laboratory tested 5 serum samples collected from patients admitted at the Nambwa health center and who were in contact with the diseased cases. They performed real-time PCR and found 2 positive results for Zaire Ebola virus. The tests were performed on 11th May and re-tested on 12th May, 2017 by the same staff. On Saturday, 13th May, the samples were re-tested by the PREDICT staff using the PREDICT protocol. They found one positive result on the 5 samples, the same that was clearly positive by real-time PCR.
Where was the laboratory testing performed (name of laboratory)?	Samples were tested at the INRB virology laboratory
Number of days between initiation of government response and lab confirmation of laboratory results.	N/A

Summary of the Outbreak or Event:	**To be filled after active outbreak or event activity has ceased**			
Working name of the outbreak:				
Total number of cases:		**Suspected:**	**Confirmed:**	**Deaths:**
	Humans			
	Domestic Animals			
	Wild Animals			
Summary of PREDICT Team response activities during the outbreak.				

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PREDICT Outbreak or Health Event Response Daily Activities/Timeline

Working Title of Investigation: *Suspicion of VHF in the Bas-Uele province, DR Congo*

*Instructions: This is the timeline of all PREDICT team activities related to this event. Please fill out in detail any PREDICT team activity as they occur on a **daily** basis (e.g., sample collection, other field activities, laboratory testing, outbreak related meetings attended, communications with the Mission or Government, etc.) in addition to the key specific items listed below.*

*Add additional rows into the specific activities listed below **in chronological order** as needed. If a specific listed event has not yet occurred, please put "pending" or "not expected" in the date column.*

Key Events:

Date	Day #	Notification or Action Taken
5/10/2017	1	First notification of 9 suspected cases of Viral Hemorrhagic Fever in the Nambwa Health Area, Likati Health Zone, Bas-Uele Province;
5/11/2017	2	PREDICT Country coordinator (CC) notified of reception of samples from the suspected cases at the INRB; PREDICT CC notified PREDICT global team
5/12/2017	3	Two samples out of five tested positive for Ebola Zaire virus, and 3 were negative by real-time PCR at the INRB virology laboratory. PREDICT CC attended the National coordination committee meeting where the Minister and his team presented the situation: 9 cases and 2 deaths, and preparations are made of an investigation team composed of epidemiologists, medical biologists and lab technicians (from the MoH and INRB) to travel tomorrow from Kinshasa to support the local team, begin contact tracing and prepare the logistic for the outbreak response. The area of Nambwa is located 45 km from Likati but it takes about 5 days to reach by car and 2 days by motorcycle. The Minister and WHO have contacted the UN Mission to provide an helicopter to bring equipment to the site. The INRB will deploy the K-Plan mobile laboratory that was purchased through the USAID funds for Yellow Fever Outbreak in Nambwa.
5/13/2017	4	PREDICT CC attended the meeting of the National coordination committee, where the Ministry of Health updated partners of the situation on the ground: a total of 11 cases were reported since the beginning of the outbreak with 3 deaths in the 3 health areas of Nambwa (7 cases and 3 deaths), Mouma (3 cases and 0 death) and Ngayi (1 case and 0 death). The provincial investigation team was back to Likati and could send this update by phone via the provincial health office.

		A team of 9 persons left Kinshasa today for Nambwa, composed of 2 epidemiologist, 1 lab technician, 1 clinician, 1 data manager, 1 information specialist, 1 hygienist, 1 logistician and 1 psychologist. They are expected to reach Nambwa on Monday or Tuesday and will prepare the logistic for the local coordination committee and begin contact tracing and sensitization. Staffs from the WHO country office and the Ministry of health are working to prepare the list of needs for the outbreak response and a budget. A request was made to the MONUSCO to provide an air lift between Kinshasa and Likati for shipping all materials and equipment, including the K-Plan mobile laboratory from the INRB.
5/15/2017	6	On Saturday, 13th May, the General Director of INRB asked PREDICT to retest the 5 samples received from the field for Filovirus using the PREDICT protocol. The reason was to have a second diagnostic method. The INRB staff tested these samples on Friday and Saturday by real time PCR, using 3 different protocols: the first targeting the L gene returned 1 positive result; the second targeting the NP gene returned 2 positive results, and the 3rd targeting the Glycoprotein gene returned 1 positive result. Using the PREDICT protocols, the PREDICT staff tested the five samples which returned only one putative positive result on the gel, from the sample which tested positive from the 3 protocols used by the INRB staff. Amplicon from this sample will be send to GATC for sequencing per our protocol. This result was as expected as the PREDICT Filovirus protocols should be and are correct for detection of this virus but are also necessarily less sensitive as a result of conserved technique, resulting in weak or negative reactions in samples with low viral load. PREDICT CC and virologist attended the National Coordination meeting. Two points were discussed: 1) the plan and budget for the outbreak response: a group from the MoH direction of disease control, the INRB, WHO, UNOCHA and UKAID finalized the plan and budget on Monday morning. Main points are: strengthening of coordination, surveillance, hygiene and biosecurity, medical and psycho-social care, laboratory diagnostic, communication and rehabilitation of health centers and the Likati General Hospital in the Bas-Uele province. No decision of quarantine will be made. The INRB will deploy two mobile laboratories, one at Nambwa (epicenter) and a second in Buta with possibility to be deployed anywhere based on the epidemiologic situation of the outbreak. The total budget for the response is $8,072,636.00 and includes:

		coordination at national, provincial and local levels ($945,377), surveillance and laboratory ($1,685,265.00), communication ($505,000.00), materials and supplies ($1,605,000.00), medical and psychosocial care ($2,313,280.00), prevention ($ 477,839.00), Water, hygiene and sanitation ($540,675). Main Challenges are: transport of goods to the affected area (THE UN may help with a Helicopter), and transport of probable cases to the Ebola Treatment Center due to bad roads. 2) the situation on the field: now the total of cases has increased to 20, reported from 4 health areas: Nambwa with 12 cases and 2 deaths, Muma with 3 cases and 1 death, Ngayi with 4 cases and 0 death, Azande with 1 case and 0 death. Samples collected will all be shipped to the INRB because the committee decided not to wait for the mobile lab to be deployed. Right now all cases are being treated at home because there is no facility for handling Ebola cases. The Ebola Treatment Center is still under rehabilitation. The team has begun to disinfect the laboratory and health centers and the local radio broadcast is used for sensitization.
5/16/2017	7	PREDICT virologist attended the National Coordination Committee. A new case was reported from Nambwa, young girl 16 years old living in a house with a suspect case. Now the total number of reported cases are 21: Nambwa 13 cases, 2 deaths; Muma 3 cases, 1 death; Ngayi 4 cases, 0 death, Azande 1 case, 0 death. 3 teams are now deployed in the field in three different locations with the following objectives : active research of suspected cases, sample collection, contacts tracing and assessment of logistic needs. A fourth team led by the Ministry of Health will leave Kinshasa tomorrow with one mobile laboratory from the INRB, prepared to perform 100 tests. WHO has mobilized PPEs from the city of Kisangani to support the response. Seven committees were set up and will be meeting everyday; PREDICT was invited to be included in the committee in charge for laboratory and research. The first meeting will be on next Thursday to analyze all needs and make request to different partners. These committees will report to the National Coordination Committee daily. PATH, a CDC Implementing Partner in charge to support the country Emergency Operation Center – GHSA is partnering with DigitalGlobe and UCLA to get precise maps of the Likati health zone. They have provided cellphones with GPS to the team who will travel to the site tomorrow.

5/17/2017	8	The PREDICT Lab manager attended the National Coordination Committee meeting at the MoH: no new cases reported from Likati, still a total of 21 cases with 3 deaths, and 4 health areas affected; samples were collected from a total of 13 cases; 5 were shipped to Kinshasa and tested at the INRB, and 8 are kept in Aketi waiting to be tested on site. The investigation team has identified a total of 416 contacts to be followed. A team from the INRB travelled this morning with the 1st mobile laboratory which will be deployed in Nambwa. The 2nd mobile laboratory (K-Plan) will be transported to the field tomorrow and will be deployed in Likati. A fourth investigation team, led by the Minister of Health will travel to the site tomorrow. WHO has confirmed that PPEs (unknown number of kits) were deployed to Aketi from their stockpile in Kisangani PREDICT was requested by the Commission of Laboratory and Research to provide for the mobile laboratory: one glovebox, 1 Qiagen extraction kit and Ethanol.
5/18/2017	9	PREDICT CC and virologist attended the 1st meeting of the commission for laboratory and research, with staffs from the INRB, CDC, UCLA and FAO-ECTAD: - The mobile lab arrived and was deployed to Aketi with 4 INRB staffs; - The K-Plan laboratory travelled today and will be deployed to Buta, the provincial capital city; - INRB transmitted a list of reagents and supplies needed to perform lab tests in the field; the list was transmitted to the MoH and FAO. The team from FAO informed that they will provide the needed supplies according to what is available now at the Central Vet Lab PREDICT virologist attended the National Coordination Committee meeting: The Minister of Health reported on his trip to Aketi: the deployed team is performing active research of suspected cases and contacts; visited health facilities and traditional healers; ongoing data collected regarding burials in villages; sensitization of local communities; different opinion leaders are intensively collaborating with investigation teams; as well as challenges due to bad roads. Epidemiological update:

		Total of 29 suspected cases reported, and 3 deaths: Nambwa Health Area=11 cases and 2 deaths; Muma Health Area=3 cases and 1 death; Ngayi Health Area=14 cases and 0 death; Azande Health Area=1 case and 0 deaths. Registered contacts under follow up = 416. A total of 35 samples collected: 5 were shipped to Kinshasa and the remaining stored at Likati waiting to be tested on site. Four new alerts received, 2 from Azande and 2 from Ngabatal, under investigation Mobile lab expected to be operational tomorrow Discussion on vaccination: Director of the Expanded Program for Immunization presented a plan and proposal for the use of experimental vaccine that was used in West Africa which is made of recombinant ZEBOV-VZV. The vaccine is efficient in protecting chimpanzees from infection. It should be conserved at -60°C, conditioned in 10 doses/vial and after reconstitution could be conserved between +2 and +8°C for a maximum of 6 hours. The vaccine is administered via intramuscular injection. The Protocol of vaccination is ready and will be submitted this evening to the Ethical Committee at KSPH for approval and will be considered a clinical trial. The vaccine is not approved to be used in humans yet. If the DRC Government accept the use of this vaccine, nearly 12,000 doses could be provided to be administered to teams working in the field.
5/19/2017	10	PREDICT virologist attended 2nd meeting of the commission for laboratory and research with staff from the INRB, CDC, UCLA: The commission has transmitted the complete list of members and partners to Ministry of Health. The General Director of INRB presented the strategy for response to the outbreak: - The Mobile Laboratory should be operational for PCR, ELISA tests and rapid tests - As there are only 3 deaths reported till today there is a possibility that this current Ebola outbreak may be mask by another unknown pathogen – INRB will also deploy a team from the Parasitology and Bacteriology Laboratories to perform investigations and diagnosis on samples collected in the field (for example recently in Banalia - Shigella and Salmonella infections were responsible for several deaths) **Reagents for diagnosis:** - Two boxes of Ebola rapid tests are available at INRB Virology

		Laboratory - Another tests will be provided by Japanese Cooperation - The Ebola tests for Mobile Laboratory (Kaplan- Prof. Parisi) were sent to DRC via DHL - The Gene Expert machine with reagents will be received this Sunday and offered by UCLA project to INRB PREDICT virologist also attended the National Coordination Committee meeting: Epidemiological update**:** At the date of May 18, 2017 a total of 32 suspected cases were reported with 4 deaths: Nambwa-11 cases, 2 deaths, Mouma – 3 cases, 1 death, Ngayi – 14 cases, 1 death*, Azande-2 cases and Ngabatala – 2 cases. Concerning the 4th death* – young girl, 22 years old died with hemorrhagic symptoms, vomiting and fever on May 8, 2017 in a small village near Ngayi. She was the family member of the 3rd died case. The burial ceremony was done for her and this was only reported when the surveillance team visited the site. Four direct contacts were identified, they are sick and under the surveillance in the village. Registered contacts: 416 persons Samples collected: 35 The Mobile Laboratory was installed and the testing of samples will start this evening. In the reference Hospital in Likati, separate room for suspected cases and sick persons was prepared for safe medical follow –up of these persons. The General Director of INRB highlighted the importance of intensive research of new cases, the daily follow-up of all contacts (two times per day with measurement of corporal temperature). He also highlighted the importance to determine the "definition of case" by the medical team deployed in the field. The follow-up of contacts is very challenging/difficult to be implemented, there is a need for trained voluntaries (ex. members of Red Cross) to help. **Vaccination Program against Ebola:** The Government has approved the use of the Ebola vaccine in DRC during this Ebola outbreak. The Protocol of vaccination was submitted to Ethical Committee at

		KSPH for approval as a clinical trial. Several scenarios were proposed and will be discussed before starting the vaccination.
5/20/2017	11	PREDICT CC attended the meeting of the commission of Laboratory and Research: Results from the CIRMF laboratory in Gabon: The 2 positive samples for Zaire Ebola Virus out of the 5 that were tested at the INRB were retested and confirmed in CIRMF. The staff at CIRMF is performing whole sequencing of the virus and will send results on Monday or Tuesday with Phylogenetic analysis. The K-Plan mobile laboratory arrived in Kisangani pending transportation to Buta, the provincial capital city. The INRB staff sent to Likati have tested 22 samples collected from suspected cases, all tests (real-time PCR) returned negative results. The director of INRB would like PREDICT to test all negative results with PREDICT protocol for the 5 PREDICT viral families. The DRC PREDICT team is unsure about this as the current sample collection is not in conformity with PREDICT protocol. PREDICT samples should be stored at -80° C soon after collection in either Trizol or VTM which is not the case on the field. PREDICT CC attended the meeting of the National Coordination Committee: The following issues were raised: The data from the field need to be cleaned, waiting for more accurate data tomorrow; the generator of the mobile laboratory is not working, and the lab is using the generator from the Health Zone office; contact tracing is challenging due to bad roads; 2 health facilities were selected to be rehabilitated and transformed to Ebola Treatment Centers (ETC). The K-Plan reagents not arrived yet at the INRB as of this evening at 4.00 PM The CDC will provide rapid tests for this outbreak It was proposed that the team in Likati prepares and sends a list of all cases and contacts, noting timeline of symptoms occurrence, date of sample collection, and clinical outcome in order to better follow the epidemiological curve and be more specific on contacts who can be

		considered to be removed from the list All commissions should prepare an operational action plan; all technical discussion should be prepared in the commissions, and each partner interested to support specific actions and activities should present this to the commission.
21/05/2017	12	-
22/05/2017	13	PREDICT CC and Virologist attended the National Coordination Committee Meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: Situation in the field: - A total of 43 suspected cases with 4 deaths: Nambwa, 24 cases and 2 deaths; Muma, 4 cases and 1 death; Ngayi 10 cases and 1 death; Azande, 3 cases and Ngabatala, 2 cases. - A total of 419 contacts registered: 158 in Nambwa, 162 in Muma, 98 in Ngayi, 1 in Azande and 0 in Ngabatala. - Number of contacts followed=54; - A total of 38 samples collected to date, of which 5 were tested at INRB and 33 being tested in the field with the Mobile laboratory in Nambwa. -- All 33 samples were negative by PCR for the Zaire Ebola virus nucleoprotein. - The K-Plan mobile laboratory that was picked up from the INRB and thought to have left for Kisangani is still in Kinshasa waiting to be transported to Buta. - The INRB team who will work on this mobile lab is already in Buta. - Dr. Pierre Rollin from CDC arrived in Kinshasa with 250 OraSure (OraQuick) rapid tests and 100 Chembio Ebola-Paludism rapid tests. These tests will be used in the field by investigation teams working at places distant from the mobile laboratory. - UCLA in partnership with Dr. Gary Kobinger (a researcher at the University of Laval, Canada, formerly with the Public Health Agency of Canada) will provide the GeneExpert to be used at the Ebola Treatment Center.
23/05/2017	14	PREDICT CC and Virologist attended 2 meetings; the meeting of the commission of Laboratory and research at INRB and the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the Commission of laboratory and research: - Sample collection from patients at the Ebola Treatment Center in Likati is ongoing. - It has been decided that 4 aliquots of each sample will be prepared: one to be tested at the mobile lab, the second to be tested using GeneExpert in the field, the third will be shipped to the CIRMF in Gabon for confirmation and the fourth will be stored at the INRB in Kinshasa.

		- The K-Plan mobile lab will be transported in Buta by a UN flight and will be installed at the Buta General Hospital. - The INRB has also received the following reagents for the GeneExpert; Filovirus and Zaire Ebola virus (2x96 tests); reagents for PCR for Ebola virus; Ebola IgM and IgG ELISA as well as reagents for Shigella, Salmonella and Malaria. 2) Current situation in the field: - A total of 48 suspected cases and 4 deaths reported: Nambwa, 28 cases and 2 deaths, Muma 5 cases and 1 death, Ngayi 10 cases and 1 death, Azande 3 cases and Ngabatala 2 cases. - A total of 419 contacts have been registered and from them 49 will be removed from the list of follow up. The remaining 370 contacts are in Nambwa: 109, Ngayi: 98, Muma: 162, Azande: 1 and Ngabatala: 0. - Radio broadcast from a local radio station is currently being used for sensitization but it needs to be improved in order for its signal to be transmitted across multiple villages. - Some staff from the Bacteriology and Parasitology labs at the INRB will travel in the days ahead to Likati to begin testing of samples for other pathogens. - At the moment, two Ebola Treatment Centers are operational; one in Likati and the other in Nambwa. They are managed by Doctors Without Borders (MSF). There is plan to set up 2 others in Muma and Ngayi.
		First specimens delivered to laboratory
		First laboratory preliminary results
		First laboratory confirmed results
		First report of results to government and taskforce
		First notification to USAID of government cleared laboratory results

In-Country Government Outbreak or Health Event Points of Contact

Public Health ministry or department:

Name:	Benoit Kebela Ilunga
Email:	kebelailunga@gmail.com
Mobile Phone:	243 (0)81 997 2691 | 243 (0)90 282 1986

Livestock ministry or department:	
Name:	Leopold Mulumba
Email:	Leopold_mulumba@yahoo.com
Mobile Phone:	243 (0)81 509 1448 | 243 (0)84 200 0178

Wildlife/Environment ministry or department:	
Name:	Jeff Mapilanga
Email:	jeffmapilanga@gmail.com
Mobile Phone:	243 (0)99 810 1924

OIE focal point:	
Name:	Honore N'Lemba Mabela
Email:	Dr_nlemba@yahoo.fr
Mobile Phone:	243 (0)81 512 6564 | 243 (0)99 990 2967

IHR focal point:	
Name:	Theophile Bokenge
Email:	drbokenge@yahoo.fr
Mobile Phone:	

FAO:	
Name:	Philippe Kone
Email:	Philippe.kone@fao.org
Mobile Phone:	243 (0)82 961 6580

WHO:	
Name:	Ernest Dabire
Email:	dabireer@who.int
Mobile Phone:	

EPT ONE HEALTH WORKFORCE Project:	
Name:	Diafuka Saila Ngita
Email:	Diafuka.saila_ngita@tufts.edu
Mobile Phone:	243 (0)81 230 4310

EPT PREPAREDNESS and RESPONSE Project:	
Name:	
Email:	
Mobile Phone:	

Other Important Contacts:

Organization:	
Name:	
Email:	
Mobile Phone:	

Organization:	
Name:	
Email:	
Mobile Phone:	

Organization:	
Name:	
Email:	
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Mobile Phone:	

Organization:	
Name:	
Email:	
Mobile Phone:	

From: Andrew Clements <aclements@usaid.gov>
To: Jonna Mazet <jkmazet@ucdavis.edu>
Sent: 5/25/2017 2:30:41 AM
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update

Thanks

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On May 25, 2017, at 2:14 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

Hello,
Today's update attached. I observe in the meeting notes that there will be 4 aliquots of each sample tested and/or stored by the listed labs. Predict is not listed. I inquired with our team, and they responded that we have not yet received a letter officially requesting our testing. It is likely that if/when that is received, we will test the sample going to INRB, where our lab is located, but that is not yet confirmed. I also asked our team to confirm how the samples that we might be asked to test will be transported (media, cold chain, etc.) and stored.
Hopefully, we'll be able to provide clarity on that in the future,
Jonna

On Tue, May 23, 2017 at 9:56 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Current update attached.
Have a nice day,
Jonna

On Sun, May 21, 2017 at 1:38 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Please see below and attached regarding what has been requested of Predict and what we believe we can offer. Note that we are concerned about both cold chain and the media into which samples are being collected in the field. We can test the samples, but there will likely be a reduction in sample quality that may impact analysis.
We will keep you posted, but please let us know if you have questions that we should pass to the in-country team.
Have a nice day,
Jonna

---------- Forwarded message ----------
From: **James Ayukekbong** <jayukekbong@metabiota.com>
Date: Sun, May 21, 2017 at 7:37 AM
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update
To: Jonna Mazet <jkmazet@ucdavis.edu>, Maria Makuwa <mmakuwa@metabiota.com>
Cc: Prime Mulembakani <pmulembakani@metabiota.com>, PREDICT-outbreak <predict-outbreak@ucdavis.edu>, Brian Bird <bhbird@ucdavis.edu>, Eddy Rubin <erubin@metabiota.com>, Karen Saylors <ksaylors@metabiota.com>, Damien Joly <djoly@metabiota.com>

Dear all,

Find attached the updated PREDICT Outbreak Rapid Report form regarding the current Ebola outbreak in DRC.

We are told the Minister of health would sign an official request for PREDICT to perform the following;

- To conduct a joined ecological research with FAO to look for Ebola virus among wild and domestic animals in Likati.

- To test all samples (including negatives) from these outbreak with the PREDICT panel.

Kind regards,

J.A Ayukekbong, PhD
Regional Coordinator /Central Africa
USAID PREDICT | Metabiota
Email: jayukekbong@metabiota.com
Mobile: +1 250-797-7755
Website: www.metabiota.com
Skype: ayukekbong.ayukepi

From: Peter Daszak <daszak@ecohealthalliance.org>
To: Jonna Mazet <jkmazet@ucdavis.edu>, Anna Willoughby <willoughby@ecohealthalliance.org>
Cc: David J Wolking <djwolking@ucdavis.edu>, "Kevin Olival, PhD" <olival@ecohealthalliance.org>
Subject: RE: URGENT: Modeling & Analytics semi-annual
Sent: Fri, 26 May 2017 17:24:14 +0000

Totally agree and Kevin knows this too.

Maybe we can talk about these on the next M&A meeting – it could be a great P2-wide project eventually, but we need to respect a student's need to get a thesis and papers….

Look forward to talking through this…

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

+1.212.380.4473 (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

From: [redacted] **On Behalf Of** Jonna Mazet
Sent: Friday, May 26, 2017 1:19 PM
To: Anna Willoughby
Cc: David J Wolking; Kevin Olival, PhD; Peter Daszak
Subject: Re: URGENT: Modeling & Analytics semi-annual

Ours is to bats, too, but limited to East Africa. I have brought this up to M&A team when I was concerned about the initial Methods, so it looks like Kevin took my advice, but we need to be careful not to scoop our own projects or people or duplicate effort.
I think we can leave the language as it is for now in the report, but we'll need to make sure we don't get cross-wise or double up internally.
More on next M&A call,
J

On Fri, May 26, 2017 at 9:23 AM, Anna Willoughby <willoughby@ecohealthalliance.org> wrote:
Hi David,

Kevin is in Thailand, so not sure if he will be able to respond this morning. I will follow up with our Tech team next week to ensure appropriate branding is visible on the EIDR site. The second item does refer to EHA work: an ongoing analysis of viral detection seasonality in bats that has been expanded significantly to include climate/life history data since we started the project in summer of 2016. Perhaps adding in that this is specific to bats will help clarify?

Let me know if you have any further questions.

Best,
Anna

On May 26, 2017, at 11:00 AM, David J Wolking <djwolking@ucdavis.edu> wrote:

Hi Kevin, Peter, and Anna,

We are getting ready to share the semi-annual report later today. Before it goes to USAID I just wanted to follow-up on a few things from your section.

1. You mention that the EIDR is a "PREDICT-derived publicly available database" and linked to it in the report. If this is accurate then we need to get some PREDICT branding on the site so that is clear to those who follow that link.
2. Also, Jonna wanted to double check that the item featured under Analyzing P-1 data refers to work in progress by UCD students and not separate efforts at EHA. For quick reference: "Finally, to assess most productive timing for sample collection, we began analysis of seasonal patterns in viral detection from PREDICT-1 data, including integration of life history data and global climate datasets"

Thanks and we appreciate a quick message back this AM if possible,

David

On Thu, May 11, 2017 at 3:26 PM, Kevin Olival, PhD <olival@ecohealthalliance.org> wrote:
David,

I'm also going to cc you when I send the full M&A M&E tomorrow, just in case you want any more detail when you're editing the SAR bullets we sent. There are figures (47 of them!) and more detailed captions in that document that may help provide some context.

Cheers,
Kevin

Kevin J. Olival, PhD
Associate Vice President for Research

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4478 (direct)
REDACTED (mobile)
1.212.380.4465 (fax)
@nycbat (twitter)
www.ecohealthalliance.org

On May 11, 2017, at 12:58 PM, David J Wolking <djwolking@ucdavis.edu> wrote:

Thanks Peter received

David

On Wed, May 10, 2017 at 6:10 PM, Peter Daszak <daszak@ecohealthalliance.org> wrote:
Hi David,

De-scientificated our M&A semi-annual following Jonna's suggestions, and added a couple of pictures….hope it's ok..

M&E stuff will come to you on Friday…

Cheers,

Peter

Peter Daszak
President

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From: Elizabeth Leasure <ealeasure@ucdavis.edu>
To: David John Wolking <djwolking@ucdavis.edu>, Amanda Fine [redacted] Brian Bird [redacted], Christine Kreuder Johnson <ckjohnson@ucdavis.edu>, Damien Joly <djoly@metabiota.com>, Eddy Rubin <erubin@metabiota.com>, Jon Epstein <epstein@ecohealthalliance.org>, "Karen Saylors" <ksaylors@metabiota.com>, Leilani Francisco <francisco@ecohealthalliance.org>, "Murray, Suzan" <MurrayS@si.edu>, "Peter Daszak" <daszak@ecohealthalliance.org>, Jonna Mazet <jkmazet@ucdavis.edu>, Woutrina A Smith <wasmith@ucdavis.edu>, Sarah Olson [redacted], "Simon Anthony" <sja2127@columbia.edu>, Tracey Goldstein <tgoldstein@ucdavis.edu>, William Karesh <karesh@ecohealthalliance.org>
Cc: Alison Andre <andre@ecohealthalliance.org>, Amanda Fuchs <fuchs@ecohealthalliance.org>, Evelyn Luciano <luciano@ecohealthalliance.org>, Megan M Doyle <mmdoyle@ucdavis.edu>, "Emma Lane" <lane@ecohealthalliance.org>, Ava Sullivan <sullivan@ecohealthalliance.org>, Taylor Elnicki <telnicki@metabiota.com>, Molly Turner <turner@ecohealthalliance.org>, Dawn Zimmerman <Zimmermand@si.edu>, "Churchill, Carolina" [redacted]
Subject: REMINDER: PREDICT EB Call: Wednesday May 31, 2017 (9-11AM PDT/12-2PM EDT)
Sent: Fri, 26 May 2017 22:34:45 +0000

Hi everyone! Just a quick reminder that we have an EB call scheduled for next Wednesday (5/31) from 9-11 am PDT (12-2 pm EDT). If you have any agenda items, please send them my way by noon on 5/30. Have a great weekend!

Thanks,
Liz

Elizabeth Leasure
One Health Institute
University of California, Davis
530-754-9034 (office)
[redacted] (cell)

From: Jonna Mazet <jkmazet@ucdavis.edu>
To: PREDICTMGT <predictmgt@usaid.gov>;Angela Wang <awang@usaid.gov>;Sarah Paige <spaige@usaid.gov>
CC: PREDICT-outbreak <predict-outbreak@ucdavis.edu>
Sent: 5/29/2017 9:32:30 AM
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update

Update attached. We will discuss and likely offer to test negatives again on Tuesday.
Hope you had a nice weekend,
Jonna

On Wed, May 24, 2017 at 5:11 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Hello,
Today's update attached. I observe in the meeting notes that there will be 4 aliquots of each sample tested and/or stored by the listed labs. Predict is not listed. I inquired with our team, and they responded that we have not yet received a letter officially requesting our testing. It is likely that if/when that is received, we will test the sample going to INRB, where our lab is located, but that is not yet confirmed. I also asked our team to confirm how the samples that we might be asked to test will be transported (media, cold chain, etc.) and stored.
Hopefully, we'll be able to provide clarity on that in the future,
Jonna

On Tue, May 23, 2017 at 9:56 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Current update attached.
Have a nice day,
Jonna

On Sun, May 21, 2017 at 1:38 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
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Skype: ayukekbong.ayukepi

PREDICT Outbreak or Health Event Rapid Report

Today's Date: *May 27th, 2017*
Working Title of Investigation: *Outbreak of Ebola Virus Disease in the Bas-Uele province, DR Congo*
Cumulative day of the outbreak investigation: 18

Please describe the disease signs and symptoms and species affected (humans, domesticated animals, wildlife:

On 8 May 2017, an alert of 9 suspected cases of Human Viral Hemorrhagic Fever and 2 deaths in the Likati Health Zone, Bas-Uele Province was received from the Provincial Health Officer. Symptoms were fever, bloody vomiting, diarrhea, and bleeding from the nose.

Location	
Country:	*Democratic Republic of Congo*
District:	*Province of Bas-Uele, Health zone of Likati, north-west of Buta*
Village/Town:	*Village in the Nambwa health area, Territory of Aketi*
GPS Coordinates (if known):	
Date that first case(s) of illness occurred (if known or estimate):	*April 22nd, 2017*
Date that PREDICT was first notified of outbreak:	*On May 10th, 2017 the PREDICT CC was informed by the INRB staff working in the virology lab that they were notified of suspected cases of VHF in the Likati Health Zone and that samples were expected to arrive for confirmatory testing anytime.* *On May 11th, 2017 the PREDICT CC was informed that the samples arrived at INRB in early afternoon and are being tested for Ebola. The same day the PREDICT CC was informed by the EPT2 focal point at the mission who talked on the phone with the Bas-Uele provincial health officer about more details on this alert: 9 cases and 2 deaths.*

Key Information	**Description of Findings/Actions/Outcomes**			
How many affected individuals?		**Suspected:**	**Confirmed:**	**Deaths:**
	Humans	47	2	4
	Domestic Animals			
	Wild Animals			
How was outbreak first noticed?	*During 16th week, a 45 year old man (case 1), fisher and farmer, became sick with fever, then bloody vomiting, bloody stools and nosebleed in the fisher camp along the river Likati,*			

	*in the Nambwa health area. He was brought to a traditional healer and then transported by moto with 2 relatives, case 2 (moto driver) and case 3 (his brother) to the Likati general hospital about 45 km away. B*ut he died on the road. Then case 3 decided to return to their village with the corpse. He was buried in the Kapayi village, Nambwa health area. On 25th April, case 2 and 3 developed the disease with same symptoms. Case 2 died the same day, and case 3 recovered. From these 3 persons, 6 other close contacts were infected. Among them, a young boy who attended the burial of case 1 died on 11th May. The provincial health office has sent a team to the site to investigate and information is expected when they return as the area has no cell phone coverage.
Where was the first reported case? What is/was the extent of geographic spread? Include comments on the apparent speed of spread.	*For now the disease is located within four health centers: Nambwa (12 cases, 2 deaths), Muma (3 cases, 1 death), Ngayi (4 cases, 0 death) and Azande (1 case, 0 death), in the Likati Health Zone, Territory of Aketi in the Bas-Uele province, where the first reported case was treated at the health center. No case is reported outside this area.*
Has the country requested support from PREDICT (include date of request)?	*Yes, the INRB General Director asked PREDICT to retest the 5 samples that were received from the field using PREDICT protocols;*
If so, which government agency requested PREDICT support?	The Ministry of Health through the INRB which is the national Public Health Laboratory
When was PREDICT response initiated (date)?	Saturday, 13th May, 2017
Are other EPT partners involved in the response (which ones and how)?	*None for now*
What type of assistance did PREDICT initially provide? Which PREDICT personnel were involved?	Testing of 5 samples from the field using PREDICT protocols and primers for Filoviruses, by the PREDICT lab manager and lab technician
When was the first official acknowledgement of the outbreak (by which government agency or other reputable body and date)? When was a response initiated and by whom? Which agencies were involved? Who was in charge of the national response?	On May 9th, 2017, the Bas-Uele provincial office informed the MoH direction of disease surveillance of the alert. A team from Buta, the provincial health office was sent to the site to investigate. A team from the MoH direction of disease control, INRB, Hygiene and the Ministry of information travelled on Saturday morning to the field. They reached Likati (health zone office) on Sunday night at 10.00 PM. On Monday morning they had a meeting with the health zone staff and sent a first report to the national coordination committee via the Ministry of Health

Was the cause of the outbreak confirmed by a laboratory? If so, give details of the initial confirmation (cause, species, specimen types tested and dates of testing if known). *Note: Daily updates for ongoing laboratory testing should be entered in the Daily Activities/Timeline table below.*	Yes, the INRB virology laboratory tested 5 serum samples collected from patients admitted at the Nambwa health center and who were in contact with the diseased cases. They performed real-time PCR and found 2 positive results for Zaire Ebola virus. The tests were performed on 11th May and re-tested on 12th May, 2017 by the same staff. On Saturday, 13th May, the samples were re-tested by the PREDICT staff using the PREDICT protocol. They found one positive result on the 5 samples, the same that was clearly positive by real-time PCR.
Where was the laboratory testing performed (name of laboratory)?	Samples were tested at the INRB virology laboratory
Number of days between initiation of government response and lab confirmation of laboratory results.	N/A

Summary of the Outbreak or Event:	**To be filled after active outbreak or event activity has ceased**			
Working name of the outbreak:				
Total number of cases:		**Suspected:**	**Confirmed:**	**Deaths:**
	Humans			
	Domestic Animals			
	Wild Animals			
Summary of PREDICT Team response activities during the outbreak.				

PREDICT Outbreak or Health Event Response Daily Activities/Timeline

Working Title of Investigation: *Suspicion of VHF in the Bas-Uele province, DR Congo*

*Instructions: This is the timeline of all PREDICT team activities related to this event. Please fill out in detail any PREDICT team activity as they occur on a **daily** basis (e.g., sample collection, other field activities, laboratory testing, outbreak related meetings attended, communications with the Mission or Government, etc.) in addition to the key specific items listed below.*

*Add additional rows into the specific activities listed below **in chronological order** as needed. If a specific listed event has not yet occurred, please put "pending" or "not expected" in the date column.*

Key Events:

Date	Day #	Notification or Action Taken
5/10/2017	1	First notification of 9 suspected cases of Viral Hemorrhagic Fever in the Nambwa Health Area, Likati Health Zone, Bas-Uele Province;
5/11/2017	2	PREDICT Country coordinator (CC) notified of reception of samples from the suspected cases at the INRB; PREDICT CC notified PREDICT global team
5/12/2017	3	Two samples out of five tested positive for Ebola Zaire virus, and 3 were negative by real-time PCR at the INRB virology laboratory. PREDICT CC attended the National coordination committee meeting where the Minister and his team presented the situation: 9 cases and 2 deaths, and preparations are made of an investigation team composed of epidemiologists, medical biologists and lab technicians (from the MoH and INRB) to travel tomorrow from Kinshasa to support the local team, begin contact tracing and prepare the logistic for the outbreak response. The area of Nambwa is located 45 km from Likati but it takes about 5 days to reach by car and 2 days by motorcycle. The Minister and WHO have contacted the UN Mission to provide an helicopter to bring equipment to the site. The INRB will deploy the K-Plan mobile laboratory that was purchased through the USAID funds for Yellow Fever Outbreak in Nambwa.
5/13/2017	4	PREDICT CC attended the meeting of the National coordination committee, where the Ministry of Health updated partners of the situation on the ground: a total of 11 cases were reported since the beginning of the outbreak with 3 deaths in the 3 health areas of Nambwa (7 cases and 3 deaths), Mouma (3 cases and 0 death) and Ngayi (1 case and 0 death). The provincial investigation team was back to Likati and could send this update by phone via the provincial health office.

		A team of 9 persons left Kinshasa today for Nambwa, composed of 2 epidemiologist, 1 lab technician, 1 clinician, 1 data manager, 1 information specialist, 1 hygienist, 1 logistician and 1 psychologist. They are expected to reach Nambwa on Monday or Tuesday and will prepare the logistic for the local coordination committee and begin contact tracing and sensitization. Staffs from the WHO country office and the Ministry of health are working to prepare the list of needs for the outbreak response and a budget. A request was made to the MONUSCO to provide an air lift between Kinshasa and Likati for shipping all materials and equipment, including the K-Plan mobile laboratory from the INRB.
5/15/2017	6	On Saturday, 13th May, the General Director of INRB asked PREDICT to retest the 5 samples received from the field for Filovirus using the PREDICT protocol. The reason was to have a second diagnostic method. The INRB staff tested these samples on Friday and Saturday by real time PCR, using 3 different protocols: the first targeting the L gene returned 1 positive result; the second targeting the NP gene returned 2 positive results, and the 3rd targeting the Glycoprotein gene returned 1 positive result. Using the PREDICT protocols, the PREDICT staff tested the five samples which returned only one putative positive result on the gel, from the sample which tested positive from the 3 protocols used by the INRB staff. Amplicon from this sample will be send to GATC for sequencing per our protocol. This result was as expected as the PREDICT Filovirus protocols should be and are correct for detection of this virus but are also necessarily less sensitive as a result of conserved technique, resulting in weak or negative reactions in samples with low viral load. PREDICT CC and virologist attended the National Coordination meeting. Two points were discussed: 1) the plan and budget for the outbreak response: a group from the MoH direction of disease control, the INRB, WHO, UNOCHA and UKAID finalized the plan and budget on Monday morning. Main points are: strengthening of coordination, surveillance, hygiene and biosecurity, medical and psycho-social care, laboratory diagnostic, communication and rehabilitation of health centers and the Likati General Hospital in the Bas-Uele province. No decision of quarantine will be made. The INRB will deploy two mobile laboratories, one at Nambwa (epicenter) and a second in Buta with possibility to be deployed anywhere based on the epidemiologic situation of the outbreak. The total budget for the response is $8,072,636.00 and includes:

		coordination at national, provincial and local levels ($945,377), surveillance and laboratory ($1,685,265.00), communication ($505,000.00), materials and supplies ($1,605,000.00), medical and psychosocial care ($2,313,280.00), prevention ($ 477,839.00), Water, hygiene and sanitation ($540,675). Main Challenges are: transport of goods to the affected area (THE UN may help with a Helicopter), and transport of probable cases to the Ebola Treatment Center due to bad roads. 2) the situation on the field: now the total of cases has increased to 20, reported from 4 health areas: Nambwa with 12 cases and 2 deaths, Muma with 3 cases and 1 death, Ngayi with 4 cases and 0 death, Azande with 1 case and 0 death. Samples collected will all be shipped to the INRB because the committee decided not to wait for the mobile lab to be deployed. Right now all cases are being treated at home because there is no facility for handling Ebola cases. The Ebola Treatment Center is still under rehabilitation. The team has begun to disinfect the laboratory and health centers and the local radio broadcast is used for sensitization.
5/16/2017	7	PREDICT virologist attended the National Coordination Committee. A new case was reported from Nambwa, young girl 16 years old living in a house with a suspect case. Now the total number of reported cases are 21: Nambwa 13 cases, 2 deaths; Muma 3 cases, 1 death; Ngayi 4 cases, 0 death, Azande 1 case, 0 death. 3 teams are now deployed in the field in three different locations with the following objectives : active research of suspected cases, sample collection, contacts tracing and assessment of logistic needs. A fourth team led by the Ministry of Health will leave Kinshasa tomorrow with one mobile laboratory from the INRB, prepared to perform 100 tests. WHO has mobilized PPEs from the city of Kisangani to support the response. Seven committees were set up and will be meeting everyday; PREDICT was invited to be included in the committee in charge for laboratory and research. The first meeting will be on next Thursday to analyze all needs and make request to different partners. These committees will report to the National Coordination Committee daily. PATH, a CDC Implementing Partner in charge to support the country Emergency Operation Center – GHSA is partnering with DigitalGlobe and UCLA to get precise maps of the Likati health zone. They have provided cellphones with GPS to the team who will travel to the site tomorrow.

5/17/2017	8	The PREDICT Lab manager attended the National Coordination Committee meeting at the MoH: no new cases reported from Likati, still a total of 21 cases with 3 deaths, and 4 health areas affected; samples were collected from a total of 13 cases; 5 were shipped to Kinshasa and tested at the INRB, and 8 are kept in Aketi waiting to be tested on site. The investigation team has identified a total of 416 contacts to be followed. A team from the INRB travelled this morning with the 1st mobile laboratory which will be deployed in Nambwa. The 2nd mobile laboratory (K-Plan) will be transported to the field tomorrow and will be deployed in Likati. A fourth investigation team, led by the Minister of Health will travel to the site tomorrow. WHO has confirmed that PPEs (unknown number of kits) were deployed to Aketi from their stockpile in Kisangani PREDICT was requested by the Commission of Laboratory and Research to provide for the mobile laboratory: one glovebox, 1 Qiagen extraction kit and Ethanol.
5/18/2017	9	PREDICT CC and virologist attended the 1st meeting of the commission for laboratory and research, with staffs from the INRB, CDC, UCLA and FAO-ECTAD: - The mobile lab arrived and was deployed to Aketi with 4 INRB staffs; - The K-Plan laboratory travelled today and will be deployed to Buta, the provincial capital city; - INRB transmitted a list of reagents and supplies needed to perform lab tests in the field; the list was transmitted to the MoH and FAO. The team from FAO informed that they will provide the needed supplies according to what is available now at the Central Vet Lab PREDICT virologist attended the National Coordination Committee meeting: The Minister of Health reported on his trip to Aketi: the deployed team is performing active research of suspected cases and contacts; visited health facilities and traditional healers; ongoing data collected regarding burials in villages; sensitization of local communities; different opinion leaders are intensively collaborating with investigation teams; as well as challenges due to bad roads. Epidemiological update:

		Total of 29 suspected cases reported, and 3 deaths: Nambwa Health Area=11 cases and 2 deaths; Muma Health Area=3 cases and 1 death; Ngayi Health Area=14 cases and 0 death; Azande Health Area=1 case and 0 deaths. Registered contacts under follow up = 416. A total of 35 samples collected: 5 were shipped to Kinshasa and the remaining stored at Likati waiting to be tested on site. Four new alerts received, 2 from Azande and 2 from Ngabatal, under investigation Mobile lab expected to be operational tomorrow Discussion on vaccination: Director of the Expanded Program for Immunization presented a plan and proposal for the use of experimental vaccine that was used in West Africa which is made of recombinant ZEBOV-VZV. The vaccine is efficient in protecting chimpanzees from infection. It should be conserved at -60°C, conditioned in 10 doses/vial and after reconstitution could be conserved between +2 and +8°C for a maximum of 6 hours. The vaccine is administered via intramuscular injection. The Protocol of vaccination is ready and will be submitted this evening to the Ethical Committee at KSPH for approval and will be considered a clinical trial. The vaccine is not approved to be used in humans yet. If the DRC Government accept the use of this vaccine, nearly 12,000 doses could be provided to be administered to teams working in the field.
5/19/2017	10	PREDICT virologist attended 2nd meeting of the commission for laboratory and research with staff from the INRB, CDC, UCLA: The commission has transmitted the complete list of members and partners to Ministry of Health. The General Director of INRB presented the strategy for response to the outbreak: - The Mobile Laboratory should be operational for PCR, ELISA tests and rapid tests - As there are only 3 deaths reported till today there is a possibility that this current Ebola outbreak may be mask by another unknown pathogen – INRB will also deploy a team from the Parasitology and Bacteriology Laboratories to perform investigations and diagnosis on samples collected in the field (for example recently in Banalia - Shigella and Salmonella infections were responsible for several deaths) **Reagents for diagnosis:** - Two boxes of Ebola rapid tests are available at INRB Virology

		Laboratory - Another tests will be provided by Japanese Cooperation - The Ebola tests for Mobile Laboratory (Kaplan- Prof. Parisi) were sent to DRC via DHL - The Gene Expert machine with reagents will be received this Sunday and offered by UCLA project to INRB PREDICT virologist also attended the National Coordination Committee meeting: Epidemiological update**:** At the date of May 18, 2017 a total of 32 suspected cases were reported with 4 deaths: Nambwa-11 cases, 2 deaths, Mouma – 3 cases, 1 death, Ngayi – 14 cases, 1 death*, Azande-2 cases and Ngabatala – 2 cases. Concerning the 4th death* – young girl, 22 years old died with hemorrhagic symptoms, vomiting and fever on May 8, 2017 in a small village near Ngayi. She was the family member of the 3rd died case. The burial ceremony was done for her and this was only reported when the surveillance team visited the site. Four direct contacts were identified, they are sick and under the surveillance in the village. Registered contacts: 416 persons Samples collected: 35 The Mobile Laboratory was installed and the testing of samples will start this evening. In the reference Hospital in Likati, separate room for suspected cases and sick persons was prepared for safe medical follow –up of these persons. The General Director of INRB highlighted the importance of intensive research of new cases, the daily follow-up of all contacts (two times per day with measurement of corporal temperature). He also highlighted the importance to determine the "definition of case" by the medical team deployed in the field. The follow-up of contacts is very challenging/difficult to be implemented, there is a need for trained voluntaries (ex. members of Red Cross) to help. **Vaccination Program against Ebola:** The Government has approved the use of the Ebola vaccine in DRC during this Ebola outbreak. The Protocol of vaccination was submitted to Ethical Committee at

		KSPH for approval as a clinical trial. Several scenarios were proposed and will be discussed before starting the vaccination.
5/20/2017	11	PREDICT CC attended the meeting of the commission of Laboratory and Research: Results from the CIRMF laboratory in Gabon: The 2 positive samples for Zaire Ebola Virus out of the 5 that were tested at the INRB were retested and confirmed in CIRMF. The staff at CIRMF is performing whole sequencing of the virus and will send results on Monday or Tuesday with Phylogenetic analysis. The K-Plan mobile laboratory arrived in Kisangani pending transportation to Buta, the provincial capital city. The INRB staff sent to Likati have tested 22 samples collected from suspected cases, all tests (real-time PCR) returned negative results. The director of INRB would like PREDICT to test all negative results with PREDICT protocol for the 5 PREDICT viral families. The DRC PREDICT team is unsure about this as the current sample collection is not in conformity with PREDICT protocol. PREDICT samples should be stored at -80° C soon after collection in either Trizol or VTM which is not the case on the field. PREDICT CC attended the meeting of the National Coordination Committee: The following issues were raised: The data from the field need to be cleaned, waiting for more accurate data tomorrow; the generator of the mobile laboratory is not working, and the lab is using the generator from the Health Zone office; contact tracing is challenging due to bad roads; 2 health facilities were selected to be rehabilitated and transformed to Ebola Treatment Centers (ETC). The K-Plan reagents not arrived yet at the INRB as of this evening at 4.00 PM The CDC will provide rapid tests for this outbreak It was proposed that the team in Likati prepares and sends a list of all cases and contacts, noting timeline of symptoms occurrence, date of sample collection, and clinical outcome in order to better follow the epidemiological curve and be more specific on contacts who can be

		considered to be removed from the list All commissions should prepare an operational action plan; all technical discussion should be prepared in the commissions, and each partner interested to support specific actions and activities should present this to the commission.
21/05/2017	12	-
22/05/2017	13	PREDICT CC and Virologist attended the National Coordination Committee Meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: Situation in the field: - A total of 43 suspected cases with 4 deaths: Nambwa, 24 cases and 2 deaths; Muma, 4 cases and 1 death; Ngayi 10 cases and 1 death; Azande, 3 cases and Ngabatala, 2 cases. - A total of 419 contacts registered: 158 in Nambwa, 162 in Muma, 98 in Ngayi, 1 in Azande and 0 in Ngabatala. - Number of contacts followed=54; - A total of 38 samples collected to date, of which 5 were tested at INRB and 33 being tested in the field with the Mobile laboratory in Nambwa. -- All 33 samples were negative by PCR for the Zaire Ebola virus nucleoprotein. - The K-Plan mobile laboratory that was picked up from the INRB and thought to have left for Kisangani is still in Kinshasa waiting to be transported to Buta. - The INRB team who will work on this mobile lab is already in Buta. - Dr. Pierre Rollin from CDC arrived in Kinshasa with 250 OraSure (OraQuick) rapid tests and 100 Chembio Ebola-Paludism rapid tests. These tests will be used in the field by investigation teams working at places distant from the mobile laboratory. - UCLA in partnership with Dr. Gary Kobinger (a researcher at the University of Laval, Canada, formerly with the Public Health Agency of Canada) will provide the GeneExpert to be used at the Ebola Treatment Center.
23/05/2017	14	PREDICT CC and Virologist attended 2 meetings; the meeting of the commission of Laboratory and research at INRB and the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the Commission of laboratory and research: - Sample collection from patients at the Ebola Treatment Center in Likati is ongoing. - It has been decided that 4 aliquots of each sample will be prepared: one to be tested at the mobile lab, the second to be tested using GeneExpert in the field, the third will be shipped to the CIRMF in Gabon for confirmation and the fourth will be stored at the INRB in Kinshasa.

		- The K-Plan mobile lab will be transported in Buta by a UN flight and will be installed at the Buta General Hospital. - The INRB has also received the following reagents for the GeneExpert; Filovirus and Zaire Ebola virus (2x96 tests); reagents for PCR for Ebola virus; Ebola IgM and IgG ELISA as well as reagents for Shigella, Salmonella and Malaria. 2) Current situation in the field: - A total of 48 suspected cases and 4 deaths reported: Nambwa, 28 cases and 2 deaths, Muma 5 cases and 1 death, Ngayi 10 cases and 1 death, Azande 3 cases and Ngabatala 2 cases. - A total of 419 contacts have been registered and from them 49 will be removed from the list of follow up. The remaining 370 contacts are in Nambwa: 109, Ngayi: 98, Muma: 162, Azande: 1 and Ngabatala: 0. - Radio broadcast from a local radio station is currently being used for sensitization but it needs to be improved in order for its signal to be transmitted across multiple villages. - Some staff from the Bacteriology and Parasitology labs at the INRB will travel in the days ahead to Likati to begin testing of samples for other pathogens. - At the moment, two Ebola Treatment Centers are operational; one in Likati and the other in Nambwa. They are managed by Doctors Without Borders (MSF). There is plan to set up 2 others in Muma and Ngayi.
24/05/2017	15	PREDICT Virologist attended 2 meetings; the meeting of the commission of Laboratory and research at INRB and the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the Commission of laboratory and research: - The commission received confirmation that the K-Plan mobile laboratory has left for Buta; - Staff from UCLA presented their results of Ebola serological survey in 4 different sites. All Ebola negative samples will be transferred to INRB for further investigation. - The field team reported new symptoms including fever and jaundice as result, it was recommended that samples be tested for Yellow Fever, Hepatitis A, B and C. 2) National coordination meeting at the MoH: - The field team revised the definition of cases, following the new case definition, there are currently 35 suspected cases and 4 deaths: Nambwa, 22 cases and 3 deaths; Muma, 3 cases and 0 death; Ngayi, 3 cases and 1 death; Azande, 3 cases and finally 2 new cases each in Mabangu and Mobenge (new sites) - A total of 294 contacts have been registered: 98 in Nambwa, 78 in

		Ngayi, 87 in Muma, 11 in Azande, 10 in Ngabatala, 4 in Mabangu and 6 in Mobenge.
25/05/2017	16	PREDICT CC and virologist attended the meeting of the commission of Laboratory and research at INRB and the virologist attended the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the Commission of laboratory and research: - All negative field samples for Ebola will be retested in Likati for other pathogens using the GeneExpert platform and in Buta using the K-Plan mobile lab (PREDICT not involved in the testing). - This testing will be for Yellow fever, Hepatitis B and Hepatitis C. An aliquot will be shipped to the INRB by a UN flight. - Two staff from the NIH in the US arrived yesterday evening and will travel to Buta and Likati tomorrow for lab support. - To ease epidemiological data interpretation, all samples shipped to the INRB are accompanied with other relevant information such as the date of disease onset, date of sample collection, signs and symptoms etc. 2) National Coordination Committee meeting: - A total of 37 suspected cases have been reported from 6 health areas, distributed as follow: - Nambwa: 20 suspected, 2 probable, 1 confirmed, 3 deaths; - Muma: 8 suspected, 1 probable and 1 confirmed; - Ngayi: 2 suspected, 1 death; - Azande: 3 suspected; - Mobenge: 2 suspected; - Mabangu: 2 suspected; - Currently, only 177 contacts are being followed: 139 out of 142 in Nambwa, 4 out of 4 in Mabangu, and 34 out of 78 in Muma. - The K-Plan mobile lab has arrived in Kisangani and will be deployed to Buta tomorrow. - Patients care and treatment for Ebola suspected cases/contacts will be free of charge in the whole of Likati health zone.
26/5/2017	17	PREDICT virologist attended the meeting of the commission of Laboratory and research at INRB. There was no National Coordination Committee meeting today **(all items are informational and do not reflect PREDICT activities)**: - The K-Plan mobile laboratory arrived in Buta, and will be deployed to the general reference hospital. Laboratory reagents for the K-Plan lab bought by INRB will be sent to Buta, including ELISA tests for HCV, HBsAg, Hepatitis E and Yellow Fever.
27/5/2017	18	**All items are informational and do not reflect PREDICT activities**:

		Situation in the field: - A total of 52 cases and 4 deaths are reported, including 47 suspected, 3 probable and 2 confirmed. - A total of 200 out of 241 registered contacts are currently being followed by the field teams: 139/142 in Nambwa, 4/4 in Mabongo, 40/78 in Muma, 11/11 in Azande and 6/6/ in Mobenge. - All 47 suspected cases tested negative for Ebola by real-time PCR in Likati. Their samples will be tested by Serology (IgM and IgG) to look for Ebola antibodies. - All field negative samples for Ebola (from suspected cases) will be transferred to INRB for further analysis. - Medical diagnostic kits will be shipped to Likati in order to support free medical care at the general hospital.
		First specimens delivered to laboratory
		First laboratory preliminary results
		First laboratory confirmed results
		First report of results to government and taskforce
		First notification to USAID of government cleared laboratory results

In-Country Government Outbreak or Health Event Points of Contact

Public Health ministry or department:	
Name:	Benoit Kebela Ilunga
Email:	kebelailunga@gmail.com
Mobile Phone:	243 (0)81 997 2691 | 243 (0)90 282 1986

Livestock ministry or department:	
Name:	Leopold Mulumba
Email:	Leopold_mulumba@yahoo.com
Mobile Phone:	243 (0)81 509 1448 | 243 (0)84 200 0178

Wildlife/Environment ministry or department:	
Name:	Jeff Mapilanga
Email:	jeffmapilanga@gmail.com
Mobile Phone:	243 (0)99 810 1924

OIE focal point:	
Name:	Honore N'Lemba Mabela
Email:	Dr_nlemba@yahoo.fr
Mobile Phone:	243 (0)81 512 6564 | 243 (0)99 990 2967

IHR focal point:	
Name:	Theophile Bokenge
Email:	drbokenge@yahoo.fr
Mobile Phone:	

FAO:	
Name:	Philippe Kone
Email:	Philippe.kone@fao.org
Mobile Phone:	243 (0)82 961 6580

WHO:	
Name:	Ernest Dabire
Email:	dabireer@who.int
Mobile Phone:	

EPT ONE HEALTH WORKFORCE Project:	
Name:	Diafuka Saila Ngita
Email:	Diafuka.saila_ngita@tufts.edu
Mobile Phone:	243 (0)81 230 4310

EPT PREPAREDNESS and RESPONSE Project:	
Name:	
Email:	
Mobile Phone:	

Other Important Contacts:

Organization:	
Name:	

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Email:	
Mobile Phone:	

Organization:	
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From: Jonna Mazet <jkmazet@ucdavis.edu>
To: Andrew Clements <aclements@usaid.gov>
CC: PREDICTMGT <predictmgt@usaid.gov>;Angela Wang <awang@usaid.gov>;Sarah Paige <spaige@usaid.gov>;PREDICT-outbreak <predict-outbreak@ucdavis.edu>
Sent: 5/31/2017 7:40:22 PM
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update

Please see the update for today and yesterday attached -- notable is that Predict will now be testing samples received using viral family protocols and is in the process of arranging MTAs for export of cDNA for deep sequencing. Support for ecological studies has been proposed/offered by Predict, FAO, and others -- see notes.
Have a nice night,
Jonna

On Wed, May 31, 2017 at 1:09 AM, Andrew Clements <aclements@usaid.gov> wrote:
Thanks. Have the ecological studies been approved and started? If not, what are the estimated dates?

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On May 31, 2017, at 1:40 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:

We are reaching out to further explore testing of the negatives, as well as providing technical assistance for the mentioned ecological studies.
Have a good night,
Jonna

On Mon, May 29, 2017 at 5:09 PM, James Ayukekbong <jayukekbong@metabiota.com> wrote:

Dear all,

Find attached the update of Ebola Virus Disease outbreak in the Bas-Uele Province in DRC as of May 29th, 2017
There are currently 19 suspected cases, 2 confirmed and 4 deaths.

Please let me know if you have any questions.

Kind regards,

J. Ayukekbong, PhD
Regional Coordinator /Central Africa
USAID PREDICT | Metabiota
Email: jayukekbong@metabiota.com
Mobile: +1 250-797-7755
Website: www.metabiota.com
Skype: ayukekbong.ayukepi

On Sat, May 27, 2017 at 7:36 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Update attached.
Have a nice weekend,
Jonna

On Wed, May 24, 2017 at 5:11 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Hello,
Today's update attached. I observe in the meeting notes that there will be 4 aliquots of each sample tested and/or stored by the listed labs. Predict is not listed. I inquired with our team, and they responded that we have not yet received a letter officially requesting our testing. It is likely that if/when that is received, we will test the sample going to INRB, where our lab is located, but that is not yet confirmed. I also asked our team to confirm how the samples that we might be asked to test will be transported (media, cold chain, etc.) and stored.
Hopefully, we'll be able to provide clarity on that in the future,
Jonna

On Tue, May 23, 2017 at 9:56 AM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Current update attached.
Have a nice day,
Jonna

On Sun, May 21, 2017 at 1:38 PM, Jonna Mazet <jkmazet@ucdavis.edu> wrote:
Please see below and attached regarding what has been requested of Predict and what we believe we can offer. Note that we are concerned about both cold chain and the media into which samples are being collected in the field. We can test the samples, but there will likely be a reduction in sample quality that may impact analysis.
We will keep you posted, but please let us know if you have questions that we should pass to the in-country team.
Have a nice day,
Jonna

---------- Forwarded message ----------
From: **James Ayukekbong** <jayukekbong@metabiota.com>
Date: Sun, May 21, 2017 at 7:37 AM
Subject: Re: [predict] [predict-outbreak] Ebola Virus Disease Outbreak in the Bas-Uele province -update
To: Jonna Mazet <jkmazet@ucdavis.edu>, Maria Makuwa <mmakuwa@metabiota.com>
Cc: Prime Mulembakani <pmulembakani@metabiota.com>, PREDICT-outbreak <predict-outbreak@ucdavis.edu>, Brian Bird <bhbird@ucdavis.edu>, Eddy Rubin <erubin@metabiota.com>, Karen Saylors <ksaylors@metabiota.com>, Damien Joly <djoly@metabiota.com>

Dear all,

Find attached the updated PREDICT Outbreak Rapid Report form regarding the current Ebola outbreak in DRC.

We are told the Minister of health would sign an official request for PREDICT to perform the following;

- To conduct a joined ecological research with FAO to look for Ebola virus among wild and domestic animals in Likati.

- To test all samples (including negatives) from these outbreak with the PREDICT panel.

Kind regards,

J.A Ayukekbong, PhD
Regional Coordinator /Central Africa
USAID PREDICT | Metabiota
Email: jayukekbong@metabiota.com
Mobile: +1 250-797-7755
Website: www.metabiota.com
Skype: ayukekbong.ayukepi

PREDICT Outbreak or Health Event Rapid Report

Today's Date: *May 31st, 2017*
Working Title of Investigation: *Outbreak of Ebola Virus Disease in the Bas-Uele province, DR Congo*
Cumulative day of the outbreak investigation: 22

Please describe the disease signs and symptoms and species affected (humans, domesticated animals, wildlife:

On 8 May 2017, an alert of 9 suspected cases of Human Viral Hemorrhagic Fever and 2 deaths in the Likati Health Zone, Bas-Uele Province was received from the Provincial Health Officer. Symptoms were fever, bloody vomiting, diarrhea, and bleeding from the nose.

Location	
Country:	*Democratic Republic of Congo*
District:	*Province of Bas-Uele, Health zone of Likati, north-west of Buta*
Village/Town:	*Village in the Nambwa health area, Territory of Aketi*
GPS Coordinates (if known):	
Date that first case(s) of illness occurred (if known or estimate):	*April 22nd, 2017*
Date that PREDICT was first notified of outbreak:	*On May 10th, 2017 the PREDICT CC was informed by the INRB staff working in the virology lab that they were notified of suspected cases of VHF in the Likati Health Zone and that samples were expected to arrive for confirmatory testing anytime.* *On May 11th, 2017 the PREDICT CC was informed that the samples arrived at INRB in early afternoon and are being tested for Ebola. The same day the PREDICT CC was informed by the EPT2 focal point at the mission who talked on the phone with the Bas-Uele provincial health officer about more details on this alert: 9 cases and 2 deaths.*

Key Information	**Description of Findings/Actions/Outcomes**			
How many affected individuals?		**Suspected:**	**Confirmed:**	**Deaths:**
	Humans	12	2	4
	Domestic Animals			
	Wild Animals			
How was outbreak first noticed?	*During 16th week, a 45 year old man (case 1), fisher and farmer, became sick with fever, then bloody vomiting, bloody stools and nosebleed in the fisher camp along the river Likati,*			

	*in the Nambwa health area. He was brought to a traditional healer and then transported by moto with 2 relatives, case 2 (moto driver) and case 3 (his brother) to the Likati general hospital about 45 km away. B*ut he died on the road. Then case 3 decided to return to their village with the corpse. He was buried in the Kapayi village, Nambwa health area. On 25th April, case 2 and 3 developed the disease with same symptoms. Case 2 died the same day, and case 3 recovered. From these 3 persons, 6 other close contacts were infected. Among them, a young boy who attended the burial of case 1 died on 11th May. The provincial health office has sent a team to the site to investigate and information is expected when they return as the area has no cell phone coverage.
Where was the first reported case? What is/was the extent of geographic spread? Include comments on the apparent speed of spread.	*For now the disease is located within four health centers: Nambwa (12 cases, 2 deaths), Muma (3 cases, 1 death), Ngayi (4 cases, 0 death) and Azande (1 case, 0 death), in the Likati Health Zone, Territory of Aketi in the Bas-Uele province, where the first reported case was treated at the health center. No case is reported outside this area.*
Has the country requested support from PREDICT (include date of request)?	*Yes, the INRB General Director asked PREDICT to retest the 5 samples that were received from the field using PREDICT protocols;*
If so, which government agency requested PREDICT support?	The Ministry of Health through the INRB which is the national Public Health Laboratory
When was PREDICT response initiated (date)?	Saturday, 13th May, 2017
Are other EPT partners involved in the response (which ones and how)?	*None for now*
What type of assistance did PREDICT initially provide? Which PREDICT personnel were involved?	Testing of 5 samples from the field using PREDICT protocols and primers for Filoviruses, by the PREDICT lab manager and lab technician
When was the first official acknowledgement of the outbreak (by which government agency or other reputable body and date)? When was a response initiated and by whom? Which agencies were involved? Who was in charge of the national response?	On May 9th, 2017, the Bas-Uele provincial office informed the MoH direction of disease surveillance of the alert. A team from Buta, the provincial health office was sent to the site to investigate. A team from the MoH direction of disease control, INRB, Hygiene and the Ministry of information travelled on Saturday morning to the field. They reached Likati (health zone office) on Sunday night at 10.00 PM. On Monday morning they had a meeting with the health zone staff and sent a first report to the national coordination committee via the Ministry of Health

Was the cause of the outbreak confirmed by a laboratory? If so, give details of the initial confirmation (cause, species, specimen types tested and dates of testing if known). *Note: Daily updates for ongoing laboratory testing should be entered in the Daily Activities/Timeline table below.*	Yes, the INRB virology laboratory tested 5 serum samples collected from patients admitted at the Nambwa health center and who were in contact with the diseased cases. They performed real-time PCR and found 2 positive results for Zaire Ebola virus. The tests were performed on 11th May and re-tested on 12th May, 2017 by the same staff. On Saturday, 13th May, the samples were re-tested by the PREDICT staff using the PREDICT protocol. They found one positive result on the 5 samples, the same that was clearly positive by real-time PCR.
Where was the laboratory testing performed (name of laboratory)?	Samples were tested at the INRB virology laboratory
Number of days between initiation of government response and lab confirmation of laboratory results.	N/A

Summary of the Outbreak or Event:	**To be filled after active outbreak or event activity has ceased**			
Working name of the outbreak:				
Total number of cases:		**Suspected:**	**Confirmed:**	**Deaths:**
	Humans			
	Domestic Animals			
	Wild Animals			
Summary of PREDICT Team response activities during the outbreak.				

PREDICT Outbreak or Health Event Response Daily Activities/Timeline

Working Title of Investigation: *Suspicion of VHF in the Bas-Uele province, DR Congo*

*Instructions: This is the timeline of all PREDICT team activities related to this event. Please fill out in detail any PREDICT team activity as they occur on a **daily** basis (e.g., sample collection, other field activities, laboratory testing, outbreak related meetings attended, communications with the Mission or Government, etc.) in addition to the key specific items listed below.*

*Add additional rows into the specific activities listed below **in chronological order** as needed. If a specific listed event has not yet occurred, please put "pending" or "not expected" in the date column.*

Key Events:

Date	Day #	Notification or Action Taken
5/10/2017	1	First notification of 9 suspected cases of Viral Hemorrhagic Fever in the Nambwa Health Area, Likati Health Zone, Bas-Uele Province;
5/11/2017	2	PREDICT Country coordinator (CC) notified of reception of samples from the suspected cases at the INRB; PREDICT CC notified PREDICT global team
5/12/2017	3	Two samples out of five tested positive for Ebola Zaire virus, and 3 were negative by real-time PCR at the INRB virology laboratory. PREDICT CC attended the National coordination committee meeting where the Minister and his team presented the situation: 9 cases and 2 deaths, and preparations are made of an investigation team composed of epidemiologists, medical biologists and lab technicians (from the MoH and INRB) to travel tomorrow from Kinshasa to support the local team, begin contact tracing and prepare the logistic for the outbreak response. The area of Nambwa is located 45 km from Likati but it takes about 5 days to reach by car and 2 days by motorcycle. The Minister and WHO have contacted the UN Mission to provide an helicopter to bring equipment to the site. The INRB will deploy the K-Plan mobile laboratory that was purchased through the USAID funds for Yellow Fever Outbreak in Nambwa.
5/13/2017	4	PREDICT CC attended the meeting of the National coordination committee, where the Ministry of Health updated partners of the situation on the ground: a total of 11 cases were reported since the beginning of the outbreak with 3 deaths in the 3 health areas of Nambwa (7 cases and 3 deaths), Mouma (3 cases and 0 death) and Ngayi (1 case and 0 death). The provincial investigation team was back to Likati and could send this update by phone via the provincial health office.

		A team of 9 persons left Kinshasa today for Nambwa, composed of 2 epidemiologist, 1 lab technician, 1 clinician, 1 data manager, 1 information specialist, 1 hygienist, 1 logistician and 1 psychologist. They are expected to reach Nambwa on Monday or Tuesday and will prepare the logistic for the local coordination committee and begin contact tracing and sensitization. Staffs from the WHO country office and the Ministry of health are working to prepare the list of needs for the outbreak response and a budget. A request was made to the MONUSCO to provide an air lift between Kinshasa and Likati for shipping all materials and equipment, including the K-Plan mobile laboratory from the INRB.
5/15/2017	6	On Saturday, 13th May, the General Director of INRB asked PREDICT to retest the 5 samples received from the field for Filovirus using the PREDICT protocol. The reason was to have a second diagnostic method. The INRB staff tested these samples on Friday and Saturday by real time PCR, using 3 different protocols: the first targeting the L gene returned 1 positive result; the second targeting the NP gene returned 2 positive results, and the 3rd targeting the Glycoprotein gene returned 1 positive result. Using the PREDICT protocols, the PREDICT staff tested the five samples which returned only one putative positive result on the gel, from the sample which tested positive from the 3 protocols used by the INRB staff. Amplicon from this sample will be send to GATC for sequencing per our protocol. This result was as expected as the PREDICT Filovirus protocols should be and are correct for detection of this virus but are also necessarily less sensitive as a result of conserved technique, resulting in weak or negative reactions in samples with low viral load. PREDICT CC and virologist attended the National Coordination meeting. Two points were discussed: 1) the plan and budget for the outbreak response: a group from the MoH direction of disease control, the INRB, WHO, UNOCHA and UKAID finalized the plan and budget on Monday morning. Main points are: strengthening of coordination, surveillance, hygiene and biosecurity, medical and psycho-social care, laboratory diagnostic, communication and rehabilitation of health centers and the Likati General Hospital in the Bas-Uele province. No decision of quarantine will be made. The INRB will deploy two mobile laboratories, one at Nambwa (epicenter) and a second in Buta with possibility to be deployed anywhere based on the epidemiologic situation of the outbreak. The total budget for the response is $8,072,636.00 and includes:

		coordination at national, provincial and local levels ($945,377), surveillance and laboratory ($1,685,265.00), communication ($505,000.00), materials and supplies ($1,605,000.00), medical and psychosocial care ($2,313,280.00), prevention ($ 477,839.00), Water, hygiene and sanitation ($540,675). Main Challenges are: transport of goods to the affected area (THE UN may help with a Helicopter), and transport of probable cases to the Ebola Treatment Center due to bad roads. 2) the situation on the field: now the total of cases has increased to 20, reported from 4 health areas: Nambwa with 12 cases and 2 deaths, Muma with 3 cases and 1 death, Ngayi with 4 cases and 0 death, Azande with 1 case and 0 death. Samples collected will all be shipped to the INRB because the committee decided not to wait for the mobile lab to be deployed. Right now all cases are being treated at home because there is no facility for handling Ebola cases. The Ebola Treatment Center is still under rehabilitation. The team has begun to disinfect the laboratory and health centers and the local radio broadcast is used for sensitization.
5/16/2017	7	PREDICT virologist attended the National Coordination Committee. A new case was reported from Nambwa, young girl 16 years old living in a house with a suspect case. Now the total number of reported cases are 21: Nambwa 13 cases, 2 deaths; Muma 3 cases, 1 death; Ngayi 4 cases, 0 death, Azande 1 case, 0 death. 3 teams are now deployed in the field in three different locations with the following objectives : active research of suspected cases, sample collection, contacts tracing and assessment of logistic needs. A fourth team led by the Ministry of Health will leave Kinshasa tomorrow with one mobile laboratory from the INRB, prepared to perform 100 tests. WHO has mobilized PPEs from the city of Kisangani to support the response. Seven committees were set up and will be meeting everyday; PREDICT was invited to be included in the committee in charge for laboratory and research. The first meeting will be on next Thursday to analyze all needs and make request to different partners. These committees will report to the National Coordination Committee daily. PATH, a CDC Implementing Partner in charge to support the country Emergency Operation Center – GHSA is partnering with DigitalGlobe and UCLA to get precise maps of the Likati health zone. They have provided cellphones with GPS to the team who will travel to the site tomorrow.

5/17/2017	8	The PREDICT Lab manager attended the National Coordination Committee meeting at the MoH: no new cases reported from Likati, still a total of 21 cases with 3 deaths, and 4 health areas affected; samples were collected from a total of 13 cases; 5 were shipped to Kinshasa and tested at the INRB, and 8 are kept in Aketi waiting to be tested on site. The investigation team has identified a total of 416 contacts to be followed. A team from the INRB travelled this morning with the 1st mobile laboratory which will be deployed in Nambwa. The 2nd mobile laboratory (K-Plan) will be transported to the field tomorrow and will be deployed in Likati. A fourth investigation team, led by the Minister of Health will travel to the site tomorrow. WHO has confirmed that PPEs (unknown number of kits) were deployed to Aketi from their stockpile in Kisangani PREDICT was requested by the Commission of Laboratory and Research to provide for the mobile laboratory: one glovebox, 1 Qiagen extraction kit and Ethanol.
5/18/2017	9	PREDICT CC and virologist attended the 1st meeting of the commission for laboratory and research, with staffs from the INRB, CDC, UCLA and FAO-ECTAD: - The mobile lab arrived and was deployed to Aketi with 4 INRB staffs; - The K-Plan laboratory travelled today and will be deployed to Buta, the provincial capital city; - INRB transmitted a list of reagents and supplies needed to perform lab tests in the field; the list was transmitted to the MoH and FAO. The team from FAO informed that they will provide the needed supplies according to what is available now at the Central Vet Lab PREDICT virologist attended the National Coordination Committee meeting: The Minister of Health reported on his trip to Aketi: the deployed team is performing active research of suspected cases and contacts; visited health facilities and traditional healers; ongoing data collected regarding burials in villages; sensitization of local communities; different opinion leaders are intensively collaborating with investigation teams; as well as challenges due to bad roads. Epidemiological update:

		Total of 29 suspected cases reported, and 3 deaths: Nambwa Health Area=11 cases and 2 deaths; Muma Health Area=3 cases and 1 death; Ngayi Health Area=14 cases and 0 death; Azande Health Area=1 case and 0 deaths. Registered contacts under follow up = 416. A total of 35 samples collected: 5 were shipped to Kinshasa and the remaining stored at Likati waiting to be tested on site. Four new alerts received, 2 from Azande and 2 from Ngabatal, under investigation Mobile lab expected to be operational tomorrow Discussion on vaccination: Director of the Expanded Program for Immunization presented a plan and proposal for the use of experimental vaccine that was used in West Africa which is made of recombinant ZEBOV-VZV. The vaccine is efficient in protecting chimpanzees from infection. It should be conserved at -60°C, conditioned in 10 doses/vial and after reconstitution could be conserved between +2 and +8°C for a maximum of 6 hours. The vaccine is administered via intramuscular injection. The Protocol of vaccination is ready and will be submitted this evening to the Ethical Committee at KSPH for approval and will be considered a clinical trial. The vaccine is not approved to be used in humans yet. If the DRC Government accept the use of this vaccine, nearly 12,000 doses could be provided to be administered to teams working in the field.
5/19/2017	10	PREDICT virologist attended 2nd meeting of the commission for laboratory and research with staff from the INRB, CDC, UCLA: The commission has transmitted the complete list of members and partners to Ministry of Health. The General Director of INRB presented the strategy for response to the outbreak: - The Mobile Laboratory should be operational for PCR, ELISA tests and rapid tests - As there are only 3 deaths reported till today there is a possibility that this current Ebola outbreak may be mask by another unknown pathogen – INRB will also deploy a team from the Parasitology and Bacteriology Laboratories to perform investigations and diagnosis on samples collected in the field (for example recently in Banalia - Shigella and Salmonella infections were responsible for several deaths) **Reagents for diagnosis:** - Two boxes of Ebola rapid tests are available at INRB Virology

		Laboratory - Another tests will be provided by Japanese Cooperation - The Ebola tests for Mobile Laboratory (Kaplan- Prof. Parisi) were sent to DRC via DHL - The Gene Expert machine with reagents will be received this Sunday and offered by UCLA project to INRB PREDICT virologist also attended the National Coordination Committee meeting: Epidemiological update**:** At the date of May 18, 2017 a total of 32 suspected cases were reported with 4 deaths: Nambwa-11 cases, 2 deaths, Mouma – 3 cases, 1 death, Ngayi – 14 cases, 1 death*, Azande-2 cases and Ngabatala – 2 cases. Concerning the 4th death* – young girl, 22 years old died with hemorrhagic symptoms, vomiting and fever on May 8, 2017 in a small village near Ngayi. She was the family member of the 3rd died case. The burial ceremony was done for her and this was only reported when the surveillance team visited the site. Four direct contacts were identified, they are sick and under the surveillance in the village. Registered contacts: 416 persons Samples collected: 35 The Mobile Laboratory was installed and the testing of samples will start this evening. In the reference Hospital in Likati, separate room for suspected cases and sick persons was prepared for safe medical follow –up of these persons. The General Director of INRB highlighted the importance of intensive research of new cases, the daily follow-up of all contacts (two times per day with measurement of corporal temperature). He also highlighted the importance to determine the "definition of case" by the medical team deployed in the field. The follow-up of contacts is very challenging/difficult to be implemented, there is a need for trained voluntaries (ex. members of Red Cross) to help. **Vaccination Program against Ebola:** The Government has approved the use of the Ebola vaccine in DRC during this Ebola outbreak. The Protocol of vaccination was submitted to Ethical Committee at

		KSPH for approval as a clinical trial. Several scenarios were proposed and will be discussed before starting the vaccination.
5/20/2017	11	PREDICT CC attended the meeting of the commission of Laboratory and Research: Results from the CIRMF laboratory in Gabon: The 2 positive samples for Zaire Ebola Virus out of the 5 that were tested at the INRB were retested and confirmed in CIRMF. The staff at CIRMF is performing whole sequencing of the virus and will send results on Monday or Tuesday with Phylogenetic analysis. The K-Plan mobile laboratory arrived in Kisangani pending transportation to Buta, the provincial capital city. The INRB staff sent to Likati have tested 22 samples collected from suspected cases, all tests (real-time PCR) returned negative results. The director of INRB would like PREDICT to test all negative results with PREDICT protocol for the 5 PREDICT viral families. The DRC PREDICT team is unsure about this as the current sample collection is not in conformity with PREDICT protocol. PREDICT samples should be stored at -80° C soon after collection in either Trizol or VTM which is not the case on the field. PREDICT CC attended the meeting of the National Coordination Committee: The following issues were raised: The data from the field need to be cleaned, waiting for more accurate data tomorrow; the generator of the mobile laboratory is not working, and the lab is using the generator from the Health Zone office; contact tracing is challenging due to bad roads; 2 health facilities were selected to be rehabilitated and transformed to Ebola Treatment Centers (ETC). The K-Plan reagents not arrived yet at the INRB as of this evening at 4.00 PM The CDC will provide rapid tests for this outbreak It was proposed that the team in Likati prepares and sends a list of all cases and contacts, noting timeline of symptoms occurrence, date of sample collection, and clinical outcome in order to better follow the epidemiological curve and be more specific on contacts who can be

		considered to be removed from the list All commissions should prepare an operational action plan; all technical discussion should be prepared in the commissions, and each partner interested to support specific actions and activities should present this to the commission.
21/05/2017	12	-
22/05/2017	13	PREDICT CC and Virologist attended the National Coordination Committee Meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: Situation in the field: - A total of 43 suspected cases with 4 deaths: Nambwa, 24 cases and 2 deaths; Muma, 4 cases and 1 death; Ngayi 10 cases and 1 death; Azande, 3 cases and Ngabatala, 2 cases. - A total of 419 contacts registered: 158 in Nambwa, 162 in Muma, 98 in Ngayi, 1 in Azande and 0 in Ngabatala. - Number of contacts followed=54; - A total of 38 samples collected to date, of which 5 were tested at INRB and 33 being tested in the field with the Mobile laboratory in Nambwa. -- All 33 samples were negative by PCR for the Zaire Ebola virus nucleoprotein. - The K-Plan mobile laboratory that was picked up from the INRB and thought to have left for Kisangani is still in Kinshasa waiting to be transported to Buta. - The INRB team who will work on this mobile lab is already in Buta. - Dr. Pierre Rollin from CDC arrived in Kinshasa with 250 OraSure (OraQuick) rapid tests and 100 Chembio Ebola-Paludism rapid tests. These tests will be used in the field by investigation teams working at places distant from the mobile laboratory. - UCLA in partnership with Dr. Gary Kobinger (a researcher at the University of Laval, Canada, formerly with the Public Health Agency of Canada) will provide the GeneExpert to be used at the Ebola Treatment Center.
23/05/2017	14	PREDICT CC and Virologist attended 2 meetings; the meeting of the commission of Laboratory and research at INRB and the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the Commission of laboratory and research: - Sample collection from patients at the Ebola Treatment Center in Likati is ongoing. - It has been decided that 4 aliquots of each sample will be prepared: one to be tested at the mobile lab, the second to be tested using GeneExpert in the field, the third will be shipped to the CIRMF in Gabon for confirmation and the fourth will be stored at the INRB in Kinshasa.

		- The K-Plan mobile lab will be transported in Buta by a UN flight and will be installed at the Buta General Hospital. - The INRB has also received the following reagents for the GeneExpert; Filovirus and Zaire Ebola virus (2x96 tests); reagents for PCR for Ebola virus; Ebola IgM and IgG ELISA as well as reagents for Shigella, Salmonella and Malaria. 2) Current situation in the field: - A total of 48 suspected cases and 4 deaths reported: Nambwa, 28 cases and 2 deaths, Muma 5 cases and 1 death, Ngayi 10 cases and 1 death, Azande 3 cases and Ngabatala 2 cases. - A total of 419 contacts have been registered and from them 49 will be removed from the list of follow up. The remaining 370 contacts are in Nambwa: 109, Ngayi: 98, Muma: 162, Azande: 1 and Ngabatala: 0. - Radio broadcast from a local radio station is currently being used for sensitization but it needs to be improved in order for its signal to be transmitted across multiple villages. - Some staff from the Bacteriology and Parasitology labs at the INRB will travel in the days ahead to Likati to begin testing of samples for other pathogens. - At the moment, two Ebola Treatment Centers are operational; one in Likati and the other in Nambwa. They are managed by Doctors Without Borders (MSF). There is plan to set up 2 others in Muma and Ngayi.
24/05/2017	15	PREDICT Virologist attended 2 meetings; the meeting of the commission of Laboratory and research at INRB and the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the Commission of laboratory and research: - The commission received confirmation that the K-Plan mobile laboratory has left for Buta; - Staff from UCLA presented their results of Ebola serological survey in 4 different sites. All Ebola negative samples will be transferred to INRB for further investigation. - The field team reported new symptoms including fever and jaundice as result, it was recommended that samples be tested for Yellow Fever, Hepatitis A, B and C. 2) National coordination meeting at the MoH: - The field team revised the definition of cases, following the new case definition, there are currently 35 suspected cases and 4 deaths: Nambwa, 22 cases and 3 deaths; Muma, 3 cases and 0 death; Ngayi, 3 cases and 1 death; Azande, 3 cases and finally 2 new cases each in Mabangu and Mobenge (new sites) - A total of 294 contacts have been registered: 98 in Nambwa, 78 in

		Ngayi, 87 in Muma, 11 in Azande, 10 in Ngabatala, 4 in Mabangu and 6 in Mobenge.
25/05/2017	16	PREDICT CC and virologist attended the meeting of the commission of Laboratory and research at INRB and the virologist attended the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the Commission of laboratory and research: - All negative field samples for Ebola will be retested in Likati for other pathogens using the GeneExpert platform and in Buta using the K-Plan mobile lab (PREDICT not involved in the testing). - This testing will be for Yellow fever, Hepatitis B and Hepatitis C. An aliquot will be shipped to the INRB by a UN flight. - Two staff from the NIH in the US arrived yesterday evening and will travel to Buta and Likati tomorrow for lab support. - To ease epidemiological data interpretation, all samples shipped to the INRB are accompanied with other relevant information such as the date of disease onset, date of sample collection, signs and symptoms etc. 2) National Coordination Committee meeting: - A total of 37 suspected cases have been reported from 6 health areas, distributed as follow: - Nambwa: 20 suspected, 2 probable, 1 confirmed, 3 deaths; - Muma: 8 suspected, 1 probable and 1 confirmed; - Ngayi: 2 suspected, 1 death; - Azande: 3 suspected; - Mobenge: 2 suspected; - Mabangu: 2 suspected; - Currently, only 177 contacts are being followed: 139 out of 142 in Nambwa, 4 out of 4 in Mabangu, and 34 out of 78 in Muma. - The K-Plan mobile lab has arrived in Kisangani and will be deployed to Buta tomorrow. - Patients care and treatment for Ebola suspected cases/contacts will be free of charge in the whole of Likati health zone.
26/5/2017	17	PREDICT virologist attended the meeting of the commission of Laboratory and research at INRB. There was no National Coordination Committee meeting today **(all items are informational and do not reflect PREDICT activities)**: - The K-Plan mobile laboratory arrived in Buta, and will be deployed to the general reference hospital. Laboratory reagents for the K-Plan lab bought by INRB will be sent to Buta, including ELISA tests for HCV, HBsAg, Hepatitis E and Yellow Fever.
27/5/2017	18	**All items are informational and do not reflect PREDICT activities**:

		Situation in the field: - A total of 52 cases and 4 deaths are reported, including 47 suspected, 3 probable and 2 confirmed. - A total of 200 out of 241 registered contacts are currently being followed by the field teams: 139/142 in Nambwa, 4/4 in Mabongo, 40/78 in Muma, 11/11 in Azande and 6/6/ in Mobenge. - All 47 suspected cases tested negative for Ebola by real-time PCR in Likati. Their samples will be tested by Serology (IgM and IgG) to look for Ebola antibodies. - All field negative samples for Ebola (from suspected cases) will be transferred to INRB for further analysis. - Medical diagnostic kits will be shipped to Likati in order to support free medical care at the general hospital.
29/05/2017	20	PREDICT Virologist attended 2 meetings; the meeting of the commission of Laboratory and research at INRB and the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the commission of Laboratory and research: - The INRB field team will begin to test samples for bacterial pathogens (e.g Shigella and Salmonella) in Buta using the K-Plan mobile lab - The field epidemiology and laboratory team began cleaning field dataset, deleting duplicates, removing all Ebola negative cases and reclassifying all remaining cases as suspected, probable, and contacts to be followed; - Testing of samples by ELISA has also began in the mobile lab in Likati - The commission thinks that it is now time to conduct ecological studies - It should be noted that a team of researchers from the University of Kisangani Center for Surveillance and Biodiversity conducted an ecological study in Likati some time before the outbreak - Investigators were told that the index case was in contact with a wild pig. Also some persons in the community reported die-offs of domestic pigs - Researchers from the NIH proposed to conduct a longitudinal study of all contacts of confirmed and probable cases to determine markers of the infection. 2) Meeting of the National Coordination Committee: - After cleaning the dataset, the field team has now reported only 19 cases and 4 deaths in total: 14 suspected cases (6 in Nambwa, 4 in Muma, 3 in Ngayi and 1 in Ngabatala); 3 probable cases (2 in Nambwa and 1 in Ngayi) and 2 confirmed cases in Nambwa; - The number of contacts registered is now 101 (20 in Nambwa; 5 in

		Mobenge; 61 in Muma and 15 in Ngayi - Die-offs of domestic pigs were reported from Azande, Ngabatala and Mobenge, and the field veterinarian team collected samples from 30 pigs and 2 goats; - The committee agreed to conduct ecological studies within the area of Aketi.
30/05/2017	21	PREDICT CC and Virologist attended the meeting of the commission of Laboratory and research at INRB. The PREDICT virologist attended the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the commission of Laboratory and research - The K-Plan mobile laboratory was successfully installed in Buta and ready to be used. The equipment will also be used for testing of bacteria pathogens in blood and stool samples. An aliquot of all negative samples will be shipped from Likati to Buta. - Two staff from the NIH arrived in Likati to support testing on the mobile laboratory platform. - Dr. Kobinger from Canada has begun testing samples in Likati by ELISA. Currently, there are 2 Ebola IgG positive samples which were negative by real-time PCR. - Aliquots of all samples negative for Ebola by real-time PCR in Likati were received at the INRB. Through support from METABIOTA, these samples will be inactivated and extracted RNA shipped to the USA for deep sequencing. The DRC PREDICT lab was also requested to test these samples using the PREDICT protocol for all priority viral families. - The local Veterinarian in Likati has collected samples from domestic pigs and goats. These samples will be shipped to the Central Veterinary Laboratory in Kinshasa for testing. FAO will also provide ELISA reagents to test for the African Swine Fever. - Ecological studies are being proposed by different institutions: PREDICT, FAO, Institut de Medecine Tropicale (Antwerp, Belgium), Robert Koch Institute (Germany), the University of Kisangani, NICD (South Africa), NIH and the University of OKAIDO (Japan). Request letters from the MoH will be released soon; all institutions are requested to send their protocols to Prof. Muyembe, director of the INRB by tomorrow. 2) National Coordination Committee meeting - Situation in the field: There are a total of 17 cases: 12 are suspected cases (6 in Nambwa, 1 in Muma, 3 in Ngayi and 2 in Azande), 3 probable cases (2 in Nambwa and 1 in Ngayi), and 2 confirmed cases (from Nambwa).

		- A total of 4 deaths: 3 from probable EVD cases (2 in Nambwa and 1 in Ngayi) and 1 from a confirmed case in Nambwa. - On the 101 contacts registered, 20 are in Nambwa, 5 in Mobenge, 61 in Muma and 15 in Ngayi. Only contacts from Nambwa and Mobenge were followed by the investigation teams. - Active surveillance activities will continue in the Likati health zone even after the declaration of the end of the outbreak.
31/05/2017	22	PREDICT CC and Virologist attended the meeting of the commission of Laboratory and research at INRB. The PREDICT virologist attended the National Coordination Committee meeting at the MoH **(all items are informational and do not reflect PREDICT activities)**: 1) Meeting of the commission of Laboratory and research - A team from the African CDC attended this meeting following the request from the government of the DRC to help set up a transboundary surveillance system with neighbor countries and to contact key actors in Ebola response management to become part of the regional collaboration center. A follow-up meeting will be held in Gabon on the 25-26 July, 2017 and the INRB is invited. The African CDC suggested that the CIRMF in Gabon be added to the list of institutions which will conduct ecological studies in the field. - In Likati a total of 4 samples tested positive for Ebola IgG by ELISA (the Elisa IgM tests are not sensitive enough). In order to interpret these results, a second sample will be collected from these 4 cases (at least 10 days after the first test) to detect any increase in antibody titer. This will enable the discrimination between recent and past infection. The field team is advised to send all clinical and epidemiological data from these 4 cases in order to support lab interpretation. - The K-Plan mobile laboratory is already in Buta. Blood and stool samples will be tested for bacteria pathogens (hemoculture and coproculture). - The first batch of Ebola negative samples from suspected cases arrived at the INRB and will be tested using the PREDICT protocol. - Metabiota DRC director will prepare an MTA for the samples that will be shipped to the USA for deep sequencing. 2) National Coordination Committee meeting - Situation in the field is unchanged, still 17 cases: 12 suspected, 3 probable and 2 confirmed, with 4 deaths and 101 contacts. - The local Vet team will travel tomorrow to Nambwa, Ngayi and Muma to investigate die-offs among domestic animals and collect samples.

		First specimens delivered to laboratory
		First laboratory preliminary results
		First laboratory confirmed results
		First report of results to government and taskforce
		First notification to USAID of government cleared laboratory results

In-Country Government Outbreak or Health Event Points of Contact

Public Health ministry or department:	
Name:	Benoit Kebela Ilunga
Email:	kebelailunga@gmail.com
Mobile Phone:	243 (0)81 997 2691 | 243 (0)90 282 1986

Livestock ministry or department:	
Name:	Leopold Mulumba
Email:	Leopold_mulumba@yahoo.com
Mobile Phone:	243 (0)81 509 1448 | 243 (0)84 200 0178

Wildlife/Environment ministry or department:	
Name:	Jeff Mapilanga
Email:	jeffmapilanga@gmail.com
Mobile Phone:	243 (0)99 810 1924

OIE focal point:	
Name:	Honore N'Lemba Mabela
Email:	Dr_nlemba@yahoo.fr
Mobile Phone:	243 (0)81 512 6564 | 243 (0)99 990 2967

IHR focal point:	
Name:	Theophile Bokenge
Email:	drbokenge@yahoo.fr

Mobile Phone:	

FAO:	
Name:	Philippe Kone
Email:	Philippe.kone@fao.org
Mobile Phone:	243 (0)82 961 6580

WHO:	
Name:	Ernest Dabire
Email:	dabireer@who.int
Mobile Phone:	

EPT ONE HEALTH WORKFORCE Project:	
Name:	Diafuka Saila Ngita
Email:	Diafuka.saila_ngita@tufts.edu
Mobile Phone:	243 (0)81 230 4310

EPT PREPAREDNESS and RESPONSE Project:	
Name:	
Email:	
Mobile Phone:	

Other Important Contacts:

Organization:	
Name:	
Email:	
Mobile Phone:	

Organization:	
Name:	
Email:	
Mobile Phone:	

Organization:	
Name:	
Email:	
Mobile Phone:	

Organization:	
Name:	
Email:	
Mobile Phone:	

Organization:	
Name:	
Email:	
Mobile Phone:	

From: [redacted]
To: Peter Daszak <daszak@ecohealthalliance.org>, Jonna Mazet <jkmazet@ucdavis.edu>, Brooke Watson <watson@ecohealthalliance.org>
Cc: David John Wolking <djwolking@ucdavis.edu>, Tracey Goldstein <tgoldstein@ucdavis.edu>, Christine Kreuder Johnson <ckjohnson@ucdavis.edu>
Subject: GVP country budget call 3pm PDT/6pm EST
Sent: Wed, 28 Jun 2017 21:20:23 +0000

Hi Peter, Jonna and Brooke,
Cc David, Tracey and Chris

Please see the call-in information below for our meeting today:

GVP country budget call June 28 3pm PDT/6pm EST

800-444-2801, Access code [redacted]

Thank you for joining,
[redacted]

[redacted] DVM MPH
One health leadership fellow
University of California, Davis
One Health Institute
School of Veterinary Medicine

From: Leilani Francisco <francisco@ecohealthalliance.org>
Sent: Fri, 4 Aug 2017 13:03:17 -0400
Subject: RE: behavioural surveillance
To: Jonna Mazet <jkmazet@ucdavis.edu>, "William B. Karesh" <karesh@ecohealthalliance.org>, Peter Daszak <daszak@ecohealthalliance.org>
Attachment

Hi all,
I'm re-sending the attachment in case it didn't go through initially.
Best,
Leilani

From: Leilani Francisco [mailto:francisco@ecohealthalliance.org]
Sent: Wednesday, July 26, 2017 2:35 PM
To: Jonna Mazet <jkmazet@ucdavis.edu>; William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>
Subject: RE: behavioural surveillance

Hi everyone,
I had a call with Amanda from the Red Cross this AM.
Please find her request attached.
It is not urgent so I can bring it up on the next EB call.
Best,
Leilani

From: Leilani Francisco [mailto:francisco@ecohealthalliance.org]
Sent: Tuesday, July 18, 2017 11:01 AM
To: 'Jonna Mazet' <jkmazet@ucdavis.edu>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>
Subject: RE: behavioural surveillance

Will do.
Best,
Leilani

From: [redacted] **On Behalf Of** Jonna Mazet
Sent: Tuesday, July 18, 2017 10:36 AM
To: Leilani Francisco <francisco@ecohealthalliance.org>
Cc: William B. Karesh <karesh@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>
Subject: Re: behavioural surveillance

Yes, please -- see what she wants and bring questions or opportunities to EB if not urgent.
Thanks,
Jonna

On Mon, Jul 17, 2017 at 11:34 AM, Leilani Francisco <francisco@ecohealthalliance.org> wrote:
Hi Jonna,
Would you like me to reach out to Amanda?
Happy to go with your preference.
Best,
Leilani

From: Andrew Clements [mailto:aclements@usaid.gov]
Sent: Saturday, July 15, 2017 9:23 AM
To: Amanda MCCLELLAND <amanda.mcclelland@ifrc.org>
Cc: Jonna Mazet <jkmazet@ucdavis.edu>; William Karesh <Karesh@ecohealthalliance.org>; francisco@ecohealthalliance.org
Subject: Re: behavioural surveillance

Hi Amanda,

I'm copying Jonna Mazet (Predict COP), Billy Karesh (Predict liaison to other EPT partners), and Leilani Francisco (Predict behavioral surveillance lead) on your request so one of them can follow up with you.

Andrew

Andrew P. Clements, Ph.D.
Senior Scientific Adviser
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On Jul 15, 2017, at 2:28 PM, Amanda MCCLELLAND <amanda.mcclelland@ifrc.org> wrote:

> Hi Andrew
> Hope you are enjoying the Geneva summer
> I wanted to follow up something you mentioned a few weeks ago n the EP3 kick off meeting. You mentioned that under the Protect project there was a behavioural surveillance component. I am interested to see how we could use behavioural surveillance and join in with our mapping team to help visualise behavioural risk. Do you have any more information about the project or a contact I could discuss with?
>
> Amanda
>
> **Amanda McClelland**
> *Health Secuirty and Risk Management*
> Community and Emergency Health Unit
> Health Department

REDACTED

From: Amanda MCCLELLAND <amanda.mcclelland@ifrc.org>
To: Leilani Francisco <francisco@ecohealthalliance.org>
Sent: 7/26/2017 11:25:46 AM
Subject: IFRC epidemic preparedness follow up

Dear Leilani,

Thank you again for the discussion today. As i mentioned we are looking to create a roster o consultants (individual or company) to support the roll out of a community and civil society focused epidemic preparedness program.

We are specifically looking for technical support to explore the idea of spatial anthropology and human geography, combined with risk mapping to inform community engagement and prevention interventions in an outbreak.

Please see the link to the current advertisement for the roster
http://reliefweb.int/job/2135774/consultantcy-epidemic-and-pandemic-preparedness-external-experts

We also plan to host a meeting to discuss the potential for this type of activity and how we would roll it out. I will send more details shortly on when this would occur.

Thank you again
amanda

From: [redacted]
To: Kevin Olival <olival@ecohealthalliance.org>
Cc: Jonna Mazet <jkmazet@ucdavis.edu>, Peter Daszak <daszak@ecohealthalliance.org>
Subject: Risk Ranking Data
Sent: Thu, 24 Aug 2017 09:48:53 +0000

Hi Kevin,

I hope you are well, congrats on the nature paper. We are making progress with the risk ranking and have pulled together a lot of the data together. I have a few pieces I would like to check with you to see if you have the data in your database before proceeding ourselves:

1. Phylogenetic distance of host species to humans using ctyB?
2. Do you have a list of host species for ICTV viruses?

Look forward to hearing from you.

Regards,

REDACTED

Project Scientist, PREDICT Project of USAID
Postdoctoral Researcher in Disease Ecology
One Health Institute
School of Veterinary Medicine
University of California Davis
1089 Veterinary Medicine Drive
Davis, CA 95616, USA

Mobile: +44 7877 818 775

From: "Kevin Olival, PhD" <olival@ecohealthalliance.org>
To: Damien Joly <djoly@metabiota.com>, Evan Eskew <eskew@ecohealthalliance.org>
Cc: Peter Daszak <daszak@ecohealthalliance.org>, Christine Kreuder Johnson <ckjohnson@ucdavis.edu>, "Dr. Jonna Mazet" <jkmazet@ucdavis.edu>, "Anna Willoughby" <willoughby@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>
Subject: Re: Proposal for PREDICT-wide M&A project
Sent: Mon, 11 Sep 2017 20:39:31 +0000

Hi Damien,
Thanks for the comments. Regarding your first point, yes, to my knowledge these are all only for P1 sequenced, confirmed, and gov't approved data.

Regarding the latter question about two positives from one animal… I'll let Evan handle that as I'm sure he's thought about this and encountered it in the analysis. Evan?

Cheers,
Kevin

Kevin J. Olival, PhD
Vice President for Research

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4478 (direct)
REDACTED
1.212.380.4465 (fax)
www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

> On Sep 11, 2017, at 4:27 PM, Damien Joly <djoly@metabiota.com> wrote:
>
> Hi Peter,
>
> This looks great from my perspective. Two minor comments:
>
> - Just to clarify, by limiting to PCR results (first sentence, top of page 2), I assume you mean those specimens with "Confirmation Result" = Positive? (i.e., not just those with a band)
> - Do you have plans on how to deal with situations where you have multiple specimens from one animal? In some initial fiddling with the P1 data, I found suggestion that an individual animal was more likely to be positive when more specimens were collected with that animal.
>
> Thanks, and sorry I'll miss the discussion this afternoon,
>
> Damien

Damien Joly, PhD
Head, Data Research
Metabiota

Assoc. Adjunct Professor · Dept. of Ecosystem and Public Health · Faculty of Vet. Med. · U. of Calgary
Information Management Coordinator · Emerging Pandemic Threats - PREDICT program

[redacted]
djoly@metabiota.com · *tel +1 250 616 4961* · *skype* damienjoly
http://www.metabiota.com

From: Peter Daszak <daszak@ecohealthalliance.org>
Sent: September 10, 2017 9:17:02 PM
To: Johnson Christine Kreuder (ckjohnson@ucdavis.edu); Jonna Mazet (jkmazet@ucdavis.edu); Damien Joly
Cc: Kevin Olival, PhD; Evan Eskew; Anna Willoughby; Aleksei Chmura
Subject: Proposal for PREDICT-wide M&A project

Dear all,

Late of course!!! Looking forward to our meeting tomorrow evening to discuss project-wide M&A activities, 5:30-6:30pm. Ahead of that, I wanted to circulate the attached proposal for the bat seasonality project. It has been a year and a half since we first agreed to start feeling this out as a global project. Evan Eskew has been leading the work here, and he reached out to Nistara and Diego (UCD PhD students) to make sure there wasn't overlap in the approach or analyses planned before putting this together and exploring the data.

I really want to get your feedback and then bring this up on Monday or Tuesday so that we can get buy-in from everyone in the room and get volunteers from across the P2 consortium for people who want to be more involved.

I know this is short notice, but the results so far are pretty straightforward. If possible please glance over before our meeting tomorrow night. Kevin will bring some hardcopies along also, if you don't have time before 5:30pm.

Cheers,

Peter

Peter Daszak
President

EcoHealth Alliance
460 West 34th Street – 17th Floor
New York, NY 10001

Tel. +1 212-380-4473
www.ecohealthalliance.org

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From:	Andrew Clements <aclements@usaid.gov>
To:	Katherine Leasure <kaleasure@ucdavis.edu>
CC:	PREDICTMGT <predictmgt@usaid.gov>;Jonna Mazet <jkmazet@ucdavis.edu>;Predict inbox <predict@ucdavis.edu>
Sent:	9/26/2017 11:43:37 PM
Subject:	Re: Change to Approved ITA - J. Ayukekbong to DRC October 2

Thanks. Will let the Mission know.

Andrew P. Clements, Ph.D.
Senior Scientific Advisor
Emerging Threats Division/Office of Infectious Diseases/Bureau for Global Health
U.S. Agency for International Development
Mobile phone: 1-571-345-4253
Email: aclements@usaid.gov

On Sep 27, 2017, at 2:29 AM, Katherine Leasure <kaleasure@ucdavis.edu> wrote:

Hi Andrew. Metabiota has submitted an amendment to the previously approved ITA for James Ayukekbong's travel to DRC (below for reference). They are requesting to move his departure date up to October 2, in order to coordinate with the funeral services of a family member that recently passed. Please let me know if you have any questions. Thank you.

Metabiota would like to request travel approval for Dr. James Ayukekbong, PREDICT Regional Coordinator for Central Africa, to travel from Yaoundé, Cameroon to Kinshasa, Democratic Republic of Congo from October 9-16,2017 to perform monitoring and evaluation of Year 3 field activities.

Trip purpose: In Kinshasa Dr. Ayukekbong will perform monitoring and evaluation of Year 3 field activities, discuss and establish strategic and operational plans for Year 4. To reduce the cost of field supplies, Dr. Ayukekbong will be carrying lab materials to the DRC team.

Katherine Leasure
HR/Payroll/Financial Assistant
One Health Institute
University of California, Davis
530-752-7526
530-752-3318 FAX
kaleasure@ucdavis.edu

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From: "Kevin Olival, PhD" <olival@ecohealthalliance.org>
To: "[redacted]"
Cc: "Dr. Jonna Mazet" <jkmazet@ucdavis.edu>, Peter Daszak <daszak@ecohealthalliance.org>
Subject: Proofs of revised EID Hotspots paper
Sent: Fri, 29 Sep 2017 19:53:08 +0000
41467_2017_923_Author.pdf
ATT00001.htm

Dear Marguerite,

Please find the attached proofs for the "Hotspots II" paper that you referenced in your email below. This is currently In Press at Nature Communications, and we hope will be out in the next 1-3 weeks.

Please let Peter or I know if you have any questions.

Cheers,
Kevin

nature COMMUNICATIONS

ARTICLE

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Global hotspots and correlates of emerging zoonotic diseases

Toph Allen[1], Kris A. Murray[2], Carlos Zambrana-Torrelio[1], Stephen S. Morse[3], Carlo Rondinini[4], Moreno Di Marco[5,6], Nathan Breit[1], Kevin J. Olival[1] & Peter Daszak[1]

Zoonoses originating from wildlife represent a significant threat to global health, security and economic growth, and combatting their emergence is a public health priority. However, our understanding of the mechanisms underlying their emergence remains rudimentary. Here we update a global database of emerging infectious disease (EID) events, create a novel measure of reporting effort, and fit boosted regression tree models to analyze the demographic, environmental and biological correlates of their occurrence. After accounting for reporting effort, we show that zoonotic EID risk is elevated in forested tropical regions experiencing land-use changes and where wildlife biodiversity (mammal species richness) is high. We present a new global hotspot map of spatial variation in our zoonotic EID risk index, and partial dependence plots illustrating relationships between events and predictors. Our results may help to improve surveillance and long-term EID monitoring programs, and design field experiments to test underlying mechanisms of zoonotic disease emergence.

[1] EcoHealth Alliance, 460 West 34th Street, 17th Floor, New York, NY 10001, USA. [2] Grantham Institute, Imperial College London, Exhibition Road, South Kensington, London SW7 2AZ, UK. [3] Mailman School of Public Health, Columbia University, 722 West 168th St #1504, New York, NY 10032, USA. [4] Global Mammal Assessment Program, Department of Biology and Biotechnologies, Sapienza University of Rome, Viale dell'Università 32, 00185 Rome, Italy. [5] ARC Centre of Excellence for Environmental Decisions, Centre for Biosiversity and Conservation Science, University of Queensland, St Lucia, QLD 4072, Australia. [6] School of Earth and Environmental Sciences, The University of Queensland, St Lucia, QLD 4072, Australia. Correspondence and requests for materials should be addressed to P.D. (email: daszak@ecohealthalliance.org)

Emerging infectious diseases (EIDs) are a significant and growing threat to global health, global economy and global security[1, 2]. Analyses of their trends suggest that their frequency and economic impact are on the rise[3, 4], yet our understanding of the causes of disease emergence is incomplete. The majority of EIDs (and almost all recent pandemics) originate in animals, mostly wildlife, and their emergence often involves dynamic interactions among populations of wildlife, livestock, and people within rapidly changing environments[5–7]. The mechanisms underlying this process are likely complex, and occur in contexts that are often characterized by a paucity of systematically collected data[8].

Global efforts to reduce the impacts of emerging diseases are largely focused on post-emergence outbreak control, quarantine, drug, and vaccine development[3]. However, delays in detection of or response to newly emerged pathogens, combined with increased global urbanization and connectivity, have resulted in recent EIDs causing extensive mortality across cultural, political, and national boundaries (e.g., HIV), and disproportionately high economic damages (e.g., SARS, H1N1). Efforts to identify the origins and causes of disease emergence at local scales, and regions from which novel diseases may be more likely to emerge, are valuable for focusing surveillance, prevention, and control programs earlier in the chain of emergence, containing EIDs closer to their source, and more effectively limiting their subsequent spread and socioeconomic impacts[8].

A previous analysis of global EID trends modeled the spatial variation of "EID events", representing records of the first appearance of a pathogen in a human population related to increased distribution (e.g., new geographic location, new host species), incidence, virulence, or other factors[4]. The EID events were divided into four groups, including wildlife origin zoonoses[4]. To model the potential risk of disease emergence, these four groups were regressed as a function of human population density and growth, latitude, rainfall, and wildlife species richness. The results suggest that wildlife origin EIDs are more likely to occur in regions with higher human population density and greater wildlife diversity (mammal species richness)[8]. However, the study is limited in its mechanistic inference due, in part, to the lack of specificity of the predictors. For example, the effect of population density could represent anthropogenic environmental changes (human pressure on landscapes), human-animal contact rates, reporting biases, or a combination of these. Furthermore, a range of potential mechanisms may not be adequately represented by this predictor set; a lack of an effect of rainfall, for example, does not discount the potential for other climatic factors to play a role, and a lack of an effect of latitude could mean that it is simply a poor proxy for other more meaningful factors that nevertheless exhibit some latitudinal variation (e.g., temperature, habitat types, biodiversity, and GDP). Improving the predictor set to better target underlying mechanisms could improve model performance and our ability to explain spatial variation in EID risk.

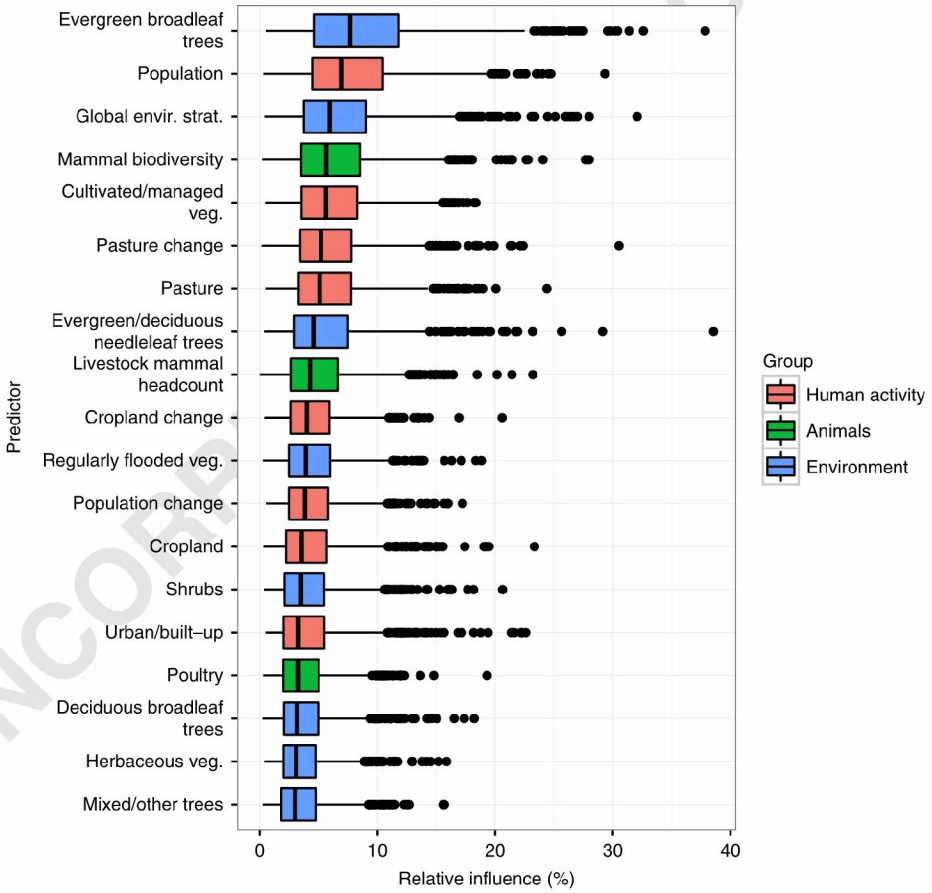

Fig. 1 The relative influence of predictors on EID event occurrence probability. The box plots show the spread of relative influence across 1000 replicate model runs to account for uncertainty in EID event location (see above). Whiskers represent the minimum or maximum datum up to 1.5 times the inter-quartile range beyond the lower or upper quartile. BRTs do not provide *p*-values or coefficients, but rank variables by their relative influence in explaining variation in the outcome[26]

The current study aims to better analyze the mechanistic underpinnings of disease emergence for zoonotic EIDs of wildlife origin, while addressing some methodological limitations of Jones et al.[4] We focus on EIDs of wildlife origin, which are responsible for nearly all recent pandemics (e.g., Ebola, MERS), constitute the majority of the high impact EIDs from the last few

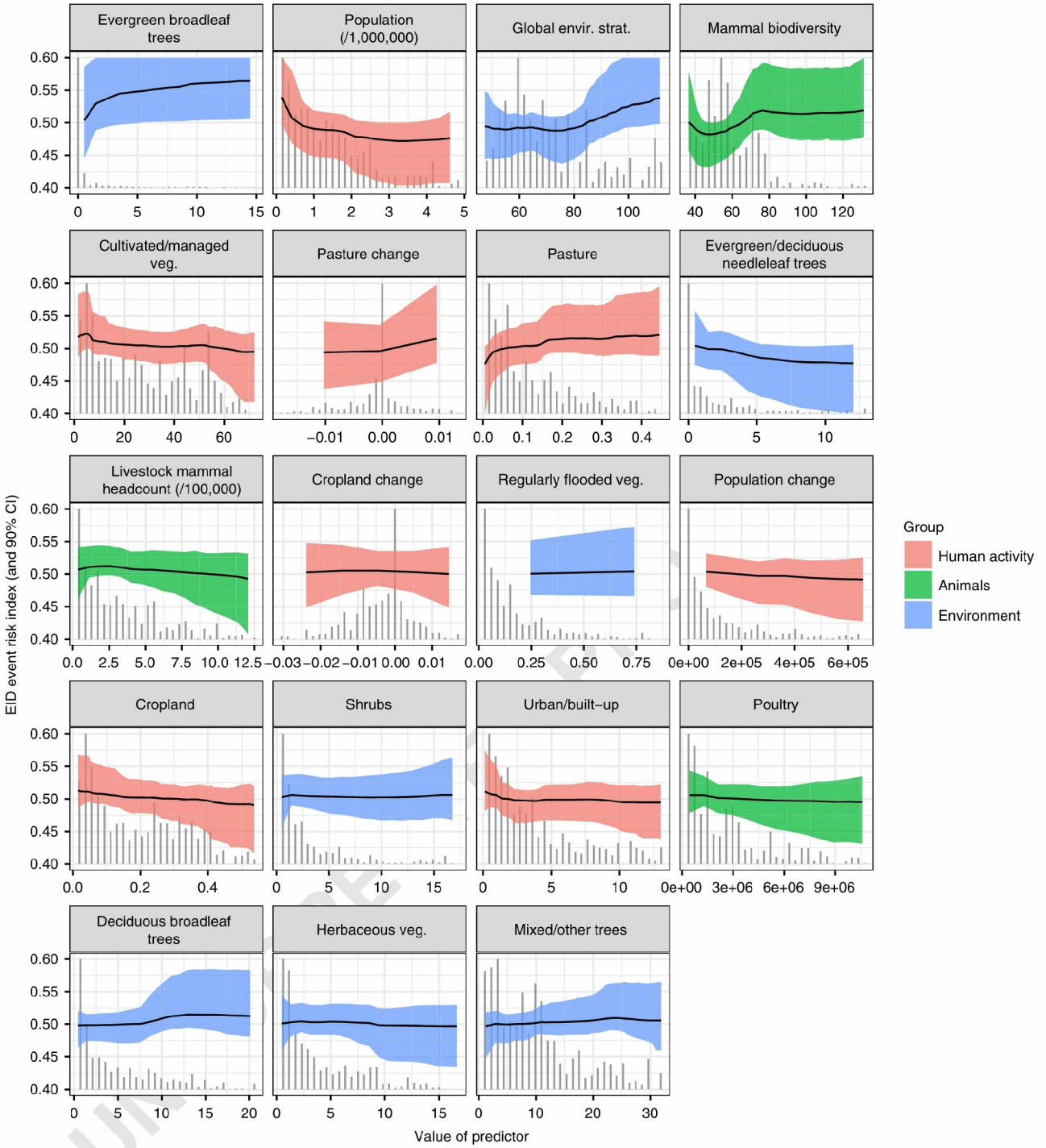

Fig. 2 Partial dependence plots showing the influence on zoonotic EID events for all predictors in the weighted boosted regression tree model, ordered by relative influence. *X* axes show the range from the 10th to 90th percentiles of sampled values of predictors (e.g., number of mammal species per grid square formammalian richness, or proportion of grid cell for a land cover type). Gray bars show histograms of predictor distribution along *X* axes. *Y* axes show the effect on the EID event risk index from that variable. Black lines show the median and colored areas show the 90% confidence intervals, computed using a bootstrap resampling regime incorporating uncertainty in EID event locations. The overall prevalence of our outcome, which indexes EID event risk, is fixed by the resampling regime between 0 and 1, with a mean at 0.5. *Y* axes are centered around the mean and scaled to 0.1 above and below. Partial dependence plots display the response for an individual variable in the model while holding all other variables constant[26, 61]. They allow a visualization of what are mostly non-linear relationships between drivers and the EID event risk index (in this case, after reporting effort is factored out.). See Supplementary Note 3 for results of the model unweighted by reporting effort

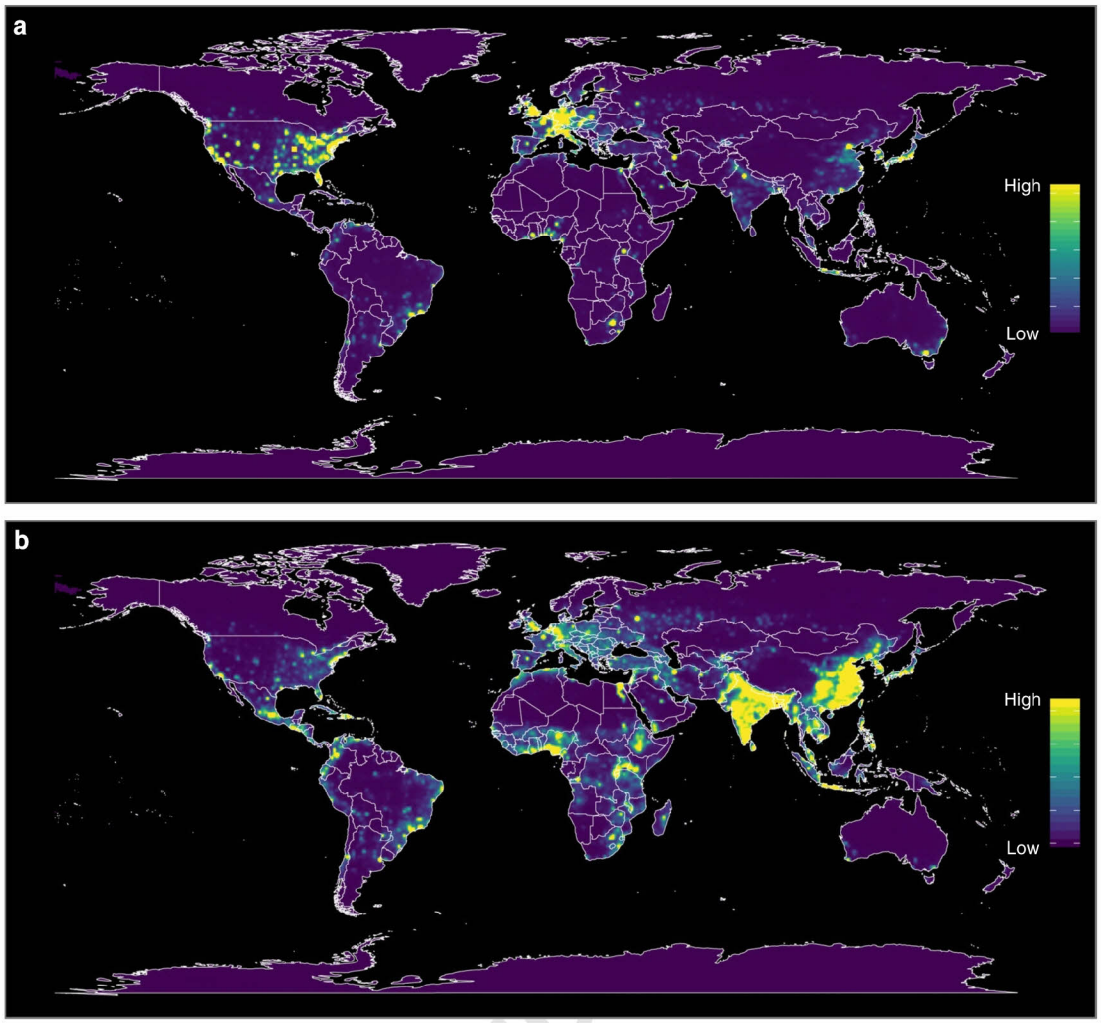

Fig. 3 Heat maps of predicted relative risk distribution of zoonotic EID events. **a** shows the predicted distribution of new events being observed (weighted model output with current reporting effort); **b** shows the estimated risk of event locations after factoring out reporting bias (weighted model output reweighted by population). See Fig. 4 for raw weighted model output. Maps were created using standard deviation scaling, with the color palette scaled to 2.5 s.d. above and below the mean

decades, and are a significantly growing proportion of all EIDs combined[4]. We updated the EID database from[4], and employed a new modeling framework (boosted regression trees, BRT) to capture high-dimensional interactions and generate response functions for individual variables. We selected a refined set of spatial predictors for their relevance to *a priori* hypotheses on plausible mechanisms underlying zoonotic EID emergence, including proxies for human activity, environmental factors, and the zoonotic pathogen pool from which novel diseases could emerge, all key features of conceptual models of zoonotic spillover[7–11]. We used an improved data set of mammal species distributions[12], and included numerous data sets on measures of land use, land-use change and land cover. Furthermore, all data sets with sufficient temporal coverage were matched to events in the EID database by decade, such that covariates more accurately reflect the prevailing conditions at the time of disease emergence. We also constructed a novel proxy of reporting effort to match the spatial resolution of the other predictors, where previous studies have relied on coarse, country-level measures, and compared EID risk predictions with and without corrections for reporting effort. Finally, we accounted for spatial uncertainty in EID event data by random resampling to explicitly take into account the difficulties of accurately geocoding EID events. Our results suggest that EID events are best predicted by the distribution of tropical forested regions, higher mammalian species richness, and variables relating to shifts in agricultural land use; and appear to occur more often in tropical regions. We identify specific areas and approaches where a research focus may identify more specific trends not apparent in our data.

Results

Variables in boosted regression tree models. After factoring out reporting effort (in the weighted model), evergreen broadleaf trees (median 7.6% of the model's predictive power), human population density (6.9%), Global Environmental Stratification (climate) (5.9%), and mammal species richness (an aspect of biodiversity) (5.6%) had the largest relative influence over the distribution of EID events (Fig. 1). Across 1000 iterations of the model, no variables consistently emerged as much stronger predictors than others but an average ranking of predictor importance could be derived. Of the top predictors, evergreen broadleaf trees (representing tropical rainforests) exhibited an overall positive trend, human population density an overall negative trend, the Global Environmental Stratification (climate)

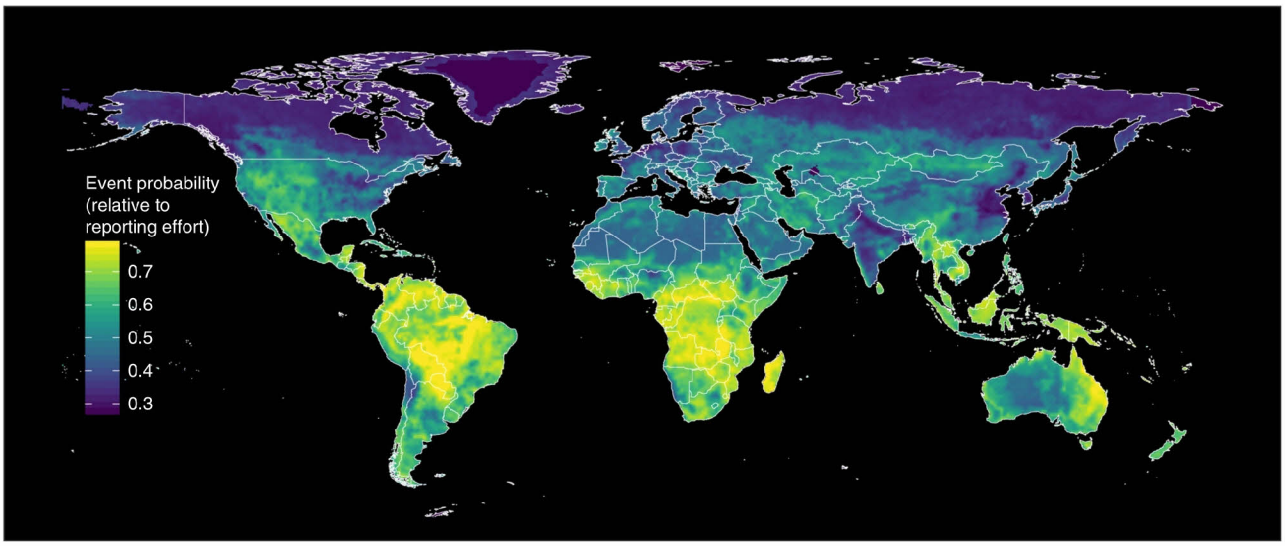

Fig. 4 Heat map of weighted model response, i.e., EID risk relative to reporting effort. Value indicates the binomial probability that a grid cell sampled at that location will contain an EID event as opposed to a background sample, when drawing equal numbers of absence and background samples weighted by reporting effort (see Methods section). This layer was weighted by reporting effort to produce the "observed" EID risk index map (Fig. 3a) and by population to produce the risk index map with bias factored out (Fig. 3b)

an idiosyncratic trend towards warmer and wetter (i.e., more tropical) climates, and mammal species richness showed an idiosyncratic trend, with higher risk values at lower and particularly higher richness values (Fig. 2). After mammal species richness, three variables involving agricultural practices followed in importance: cultivated/managed vegetation (5.6%), pasture change (5.2%), and areas dedicated to pasture (5.1%). In the unweighted model, which did not account for reporting effort (Supplementary Note 3), urban/built-up land was by far the strongest predictor of observed events, explaining a median of 30.6% of the model's variation and exhibiting a distinct positive trend.

Global distribution of EID risk index. Relative to the observed risk index for EID events, the model's estimated risk index correcting for reporting bias (Fig. 3) is more concentrated in tropical regions. Areas of higher suitability for EID occurrence are fairly evenly distributed across the continents, with no major land mass free from areas predicted to be suitable for EIDs. In particular, areas of high population outside the tropics, such as cities in Europe, the United States, Asia and Latin America remain among areas at the high end of the risk index. Tropical regions in North America, Asia, Central Africa, and regions of South America have more extensive areas of predicted EID occurrence.

Model performance and validation statistics. Our model validation statistics were computed both for the weighted model —with a background, or absence, sample weighted by reporting effort, effectively computing statistics on the residuals of that variable—and our unweighted model, using a background sample uniform across land area. The weighted bootstrap model reported a median of 31.6% of deviance explained across the 1000 replicate models (empirical 90% confidence interval (CI) 15.9% to 50.5%), whereas the unweighted model explained a median 50.2% of deviance (empirical 90% CI 35.8% to 67.2%). Our weighted model's cross-validation statistics, computed over 100 runs of 10-fold cross-validation, varied depending on the weighting of the null validation sample. With validation absences weighted by reporting effort, the weighted model had a median AUC of 0.64, with an empirical 90% confidence interval ranging from 0.54 to 0.69 (out of possible values between 0 and 1, with 0.5 indicating performance no better than random). The median True Skill Statistic (TSS) was 0.23 with an empirical 90% CI of 0.14 to 0.33 (out of a range of −1 to 1). These indicate low to moderate predictive performance[13–15]. Evaluated against an unweighted null, the weighted model had a median AUC of 0.78 (90% CI (0.75, 0.81)) and a median TSS of 0.43 (90% CI (0.37, 0.50)). The unweighted model evaluated against to an unweighted null, had a median AUC of 0.77 (90% CI (0.73, 0.81)) and a median TSS of 0.44 (90% CI (0.37, 0.50)).

Discussion

We developed a spatial model to describe the global spatial patterns of zoonotic EIDs. Our main model (the "weighted model" factored out clear effects of reporting effort, which otherwise biases our ability to interpret EID event observations. It ranked risk factors according to their predictive power, capturing both their main effects and potential interactions with other variables, and we derived the directionality and shape of their relationships to EID events for graphical interpretation. Our results suggest that the risk of disease emergence is elevated in tropical forest regions, high in mammal biodiversity, and experiencing anthropogenic land use changes related to agricultural practices[16–18].

The link between mammal biodiversity and zoonotic disease emergence has been identified previously[4] and hypothesized widely[8, 19]. Areas with tropical forest and high mammalian biodiversity were elevated on our EID risk index (henceforth "EID risk"), although the uncertainty of the estimates was high. It may be that these variables represent the same mechanism, as tropical forests are generally areas of high biodiversity[20], and the apparent association may be attenuated by the presence of both in the model. This trend is consistent with existing hypotheses, which suggest greater host biodiversity, increases the "depth" of the pathogen pool from which novel pathogens may emerge, which in turn increases the potential for novel zoonotic pathogens to emerge[21]. There is a large literature on the relationship between biodiversity and infectious disease risk in people, with some studies suggesting that high host

biodiversity decreases risk or that biodiversity loss may increase risk (i.e., the dilution effect)[22], while others refute the generalizability of this[23, 24] or suggest disease richness or prevalence increases with increasing wildlife species richness[13]. Our findings look at the global scale and a large group of pathogens, and so do not speak directly to this debate: although the dominant trend is an increase in risk of disease emergence with higher mammalian richness, this neither rules out nor substantiates the possibility of a dilution effect for specific diseases. Rather, it is consistent with previous suggestions that the relationship between biodiversity and disease risk is complex, context-specific and idiosyncratic[23].

When not accounting for reporting effort (unweighted), our model showed urban land as having a very strong positive association with EID events. However, this can be interpreted as an effect of reporting bias, since (1) urban land was also strongly associated with our measure of reporting effort, and (2) fitting our weighted model, relative to reporting effort, attenuated this effect. Similarly, although population density was not found to be an important predictor in the unweighted model (median relative influence 2.2%), weighting the model by reporting effort drove up its importance (median rel. inf. 6.9%), such that EID risk was inversely related to population density. Population density was also included in the reporting effort model, but was not as strong a predictor (rel. inf. 3.6%) as urban land (rel. inf. 45.2%). Theoretically, population has a baseline multiplicative effect on human disease events[25]—of which EID events are a subclass—and their detection is modulated by reporting effort. Reporting effort appears to be associated with urbanization, but reporting effort and urbanization are also both products of human population. We did not attempt to fully disentangle these factors, instead using our measure of reporting effort to present a map of emerging infectious disease hotspots with bias "factored out" (described below in Methods section).

Our reporting effort measure was created by matching place names in a subset of the biomedical literature. The BRT model of reporting effort model suggested that the distribution of this effort was strongly and positively related to urban areas. This could be because our extraction of place names biases the outcome toward urban areas, or it may accurately represent the true distribution of reporting toward urban areas, or a combination of the two. In either case, our reporting effort data set is likely to be a large improvement over similar previous studies that have used country-level data to control heterogeneous reporting effort in better-than country-level spatial analyses of disease risk[4, 25] (detailed fully in Supplementary Methods).

The work presented here builds on previous research[4] in a number of important ways to advance our understanding of wildlife origin zoonotic disease emergence. First, our model building approach explores the explanatory value of a large collection of globally gridded data on environmental, demographic, and host diversity variables, including newly developed models of mammal distributions and richness patterns. This has allowed us to close the gap between predictors and a priori mechanistic hypotheses specifically relevant to zoonotic disease emergence from wildlife reservoirs. Second, we adopted a machine-learning modeling approach (boosted regression trees) suited to the analysis of complex ecological data[26], and used various resampling regimes to measure and visualize multiple sources of uncertainty (model uncertainty, spatial uncertainty of EID events, and temporal uncertainty of covariates matching with events) and predictive performance. Third, we have attempted to improve how the model accounts for uneven global distribution of surveillance and research on disease event detection (i.e., report effort). This includes an algorithm-based approach to more realistically map reporting effort and shows the significant implications that a finer-scale, sub-national resolution variable for reporting effort can have for a model. Finally, we were able to temporally match predictors to events.

Despite using a more flexible modeling framework, there are limitations to our approach. When differentiating between EID events and a uniformly weighted background sample, our weighted and unweighted models had an AUC of 0.78 and 0.77, and a TSS of 0.43 and 0.41, respectively, indicating moderate predictive performance. However, against a background sample weighted by reporting effort, our weighted model had an AUC of 0.61 and a TSS of 0.18, indicating low–moderate performance. These statistics indicate much unexplained variation. While broad changes in zoonotic EID relative risk are evident in the partial dependence plots, in areas of elevated risk CIs are generally wide enough that quantitative relationships remain uncertain.

Wherever possible, we tried to define and incorporate uncertainty into our model (e.g., correcting for uncertainty in location by sampling EID events from within known areas of occurrence, and correcting for literature-level biases by weighting background samples by our measure of observation effort). Multiple factors contribute to this uncertainty. First, analyses were conducted using gridded data at 1° WGS84 resolution (c. 100 km at the equator), the same resolution used previously[4]. Our choice of resolution for predictor data sets was constrained by data availability, since all were downscaled to the lowest common spatial resolution. Second, CIs are widest in regions for each variable where fewer grid cells were sampled. Since our weighted model sampled fewer grid cells proportional with reporting effort, these represent areas where more reporting effort —including ground-truthing studies—may increase confidence. Third, another limitation shared with ref. [4] is the underlying accuracy and suitability of EID event data, which were drawn from a review of published literature. Individual studies carry their own biases, inaccuracies, and different approaches to collecting and documenting data, and this alone adds an unknown amount of imprecision and potential bias to our outcome data set. Finally, our goal of creating a single model, to look for common trends in emerging wildlife origin zoonotic diseases, likely imposes limitations on the specificity of trends we can examine. In reality, different classes of diseases (e.g., viruses versus bacteria) and indeed individual diseases have their own unique biology and ecology, with different drivers and sets of conditions being more or less important in shaping the emergence process[27]. Because of these limitations, we refrain from making specific (e.g., city by city) interpretations of the model's output, rather noting broad trends in geographic regions and environment types of intererest.

Wide confidence intervals in areas of elevated EID risk suggest areas for future study, and underscore the need for targeted long-term disease surveillance and monitoring in these areas. Collection of more accurate spatiotemporal data on events surrounding disease emergence, including initial emergence events, using a combination of large scale field research (e.g., USAID's PREDICT project[28]) and digital disease detection tools[29] would help alleviate this issue in the future by generating more consistent data on a larger scale, potentially automatically[30]. These data sets will aid efforts to better define the point at which a disease becomes "emerging", and allow the programmatic definition and examination of different definitions of emergence (e.g., first appearance vs. increasing incidence, etc.) in testable form[31].

Future work may be able to enhance the predictive power of this approach by focusing on even tighter classes of disease, taxonomic groups of pathogens and hosts, or transmission modes, and building models to forecast changes in risk

Table 1 List of predictor layers included in the model

Variable	Unit per grid cell	Type	Source data set	Processing	Temporal resolution
Human population	Population	Human activity	GRUMP	Rescaled	Decadal
Population change	Change in population	Human activity	GRUMP (calculated)	Calculated from rescaled layers	Decadal
Cropland	Proportion	Human activity	HYDE	Rescaled	Decadal
Cropland change	Change in proportion	Human activity	HYDE (calculated)	Calculated from rescaled layers	Decadal
Pasture	Proportion	Human activity	HYDE	Rescaled	Decadal
Pasture change	Change in proportion	Human activity	HYDE (calculated)	Calculated from rescaled layers	Decadal
Urban land	Percentage	Human activity	EarthEnv	Rescaled	Decadal
Managed/cultivated vegetation	Percentage	Human activity	EarthEnv	Rescaled	Static
Mammalian species richness	Count of species	Animals/hosts	Global Mammal Assessment	Reprojected, rescaled	Static
Domestic mammal headcount	Count of animals	Animals/hosts	GLW	Rescaled, summed buffalo, cattle, goat, pig, sheep headcounts	Static
Poultry headcount	Count of animals	Animals/hosts	GLW	Rescaled	Static
Global environmental stratification	Global environmental stratification	Environment	GEnS	Rescaled	Static
Evergreen/deciduous needleleaf trees	Percentage	Environment	EarthEnv	Rescaled	Static
Evergreen broadleaf trees	Percentage	Environment	EarthEnv	Rescaled	Static
Deciduous broadleaf trees	Percentage	Environment	EarthEnv	Rescaled	Static
Mixed/other trees	Percentage	Environment	EarthEnv	Rescaled	Static
Shrubs	Percentage	Environment	EarthEnv	Rescaled	Static
Herbaceous vegetation	Percentage	Environment	EarthEnv	Rescaled	Static
Regularly flooded vegetation	Percentage	Environment	EarthEnv	Rescaled	Static
Reporting effort	Weighted number of mentions in publications	Observation bias	(Internal)	(See methods)	Static

distribution or to examine more specific mechanistic hypotheses. For example, our model includes a single layer representing total mammal species richness, whereas recent work has shown that the number of zoonotic viruses varies across mammal species and taxa[32]. Efforts to examine the commonalities of disease emergence may benefit from incorporating host-specific or disease-specific models in a hierarchical approach, allowing certain parameters to vary across diseases, disease classes, or other properties.

Despite shortcomings, our improvements to the earlier model allowed us to find quantitative support for previously only hypothesized factors that increase the risk of EID events. Our findings, therefore, have broad implications for surveillance, monitoring, control, and research on emerging infectious diseases. Like Jones et al.,[4] we find that EID events are observed predominantly in developed countries, where surveillance is strongest, but that our predicted risk is higher in tropical, developing countries.

Our spatial mapping has direct relevance to ongoing surveillance and pathogen discovery efforts[33]. It shows that the global distribution of zoonotic EID risk (and the presence of EID "hotspots") is concentrated in tropical regions where wildlife biodiversity is high and land-use change is occurring. These regions are likely to be the most cost effective for surveillance programs targeting wildlife, livestock or people for novel zoonoses, and for pandemic prevention programs that build capacity and infrastructure to pre-empt and control outbreaks[28]. Further honing the EID risk index within regions and countries might also inform the planning of large land-use change programs such as logging and mining concessions, dam-building, and road development[34]. These activities carry an intrinsic risk of disease emergence by increasing human or livestock contact with wildlife in new regions or by disrupting disease dynamics in reservoir hosts[21, 35], and have been repeatedly linked to outbreaks of novel EIDs.

Similarly, the partial dependence plots allow a deeper understanding of the largely non-linear relationships between EID drivers and disease emergence that can be used to design field experiments to test specific and generalizable hypotheses on the drivers of zoonotic disease emergence. These should include field sites along land use gradients within EID hotspot countries where controlled sampling protocols are used to identify how wildlife biodiversity, known and unknown pathogen diversity (e.g., using viral family level degenerate primers for PCR[36]), and human contact with wildlife varies across a landscape. Such an approach will provide a way to identify the fine-scale rules that govern disease emergence and provide a richer understanding of what drives EID risk on-the-ground, a critical extension of this modeling approach.

Methods

Zoonotic EID events as response variable. We followed the definition of an emerging infectious disease and an EID event used in ref. [4]—specifically, events documented in the scientific literature denoting the first emergence of pathogen in a human population where that pathogen was classified as "emerging" due to recent spillover from an animal reservoir, a significant increase in its incidence or geographic distribution in the human population, a marked change in its pathogenicity or virulence, or other factors. In this study we focus only on EID events of wildlife origin ("wildlife zoonoses") because these represent the majority of EID events in the most recent decade studied, are increasing significantly as a proportion of all EIDs after correcting for reporting bias, include most of the highest impact EIDs of recent decades (e.g., Ebola viruses, Nipah virus) and almost all recent pandemics (e.g., pandemic influenza viruses, SARS). Data on EID events were derived from an updated version of the database originally used by ref. [4] (Supplementary Data 1), which contained EID events ranging from 1940 to 2004 ($n = 335$ total, $n = 145$ for wildlife zoonoses (43.3% of all EIDs)). We updated the database to include EID events for wildlife zoonoses through 2008 ($n = 224$), following the methodology in ref. [4] so as to include only diseases reported in the peer-reviewed literature, where there is evidence that a disease is emerging for one of the reasons laid out above. In addition, we only included the first emergence of a new disease-causing agent, such that the MERS Coronavirus was included, but not reports of new strains of Ebola virus. For each EID event, data were derived from the literature, if available, for date, location (see below), pathogen genus and species, zoonotic origin and type, and associated or hypothesized drivers, following ref. [4]. Location data for initial EID emergence events were variable in their geographic specificity, ranging from precise coordinates to broader regions (e.g., municipalities, counties, districts) or entire continents depending on details reported in the primary literature. A spatial polygon was created for each event that represented the most precise municipal region the EID event was known to have occurred in. All EID event polygons, regardless of precision, were included in our bootstrap resampling framework; removing those with geographic uncertainty (e.g., those with only country-level resolution) may artificially inflate the apparent certainty of our model, and our resampling scheme limits their impact to appropriate levels. Events with precise coordinates were also assigned a polygon for consistency of data format, but rather than using a municipal boundary, the event was assigned a 5 km circular buffer zone. EID polygons were subsampled for model fitting as described below. Because our model matches EID events with decadal population and land use data (described below), we restricted our analyses to decades for which covariate data exist, excluding events before 1970 and leaving $n = 147$ records for analysis (66% of wildlife zoonosis events).

Explanatory variables. We compiled spatial data layers for 20 predictors in four broad categories to decompose which factors are associated with zoonotic disease emergence. These reflected the most frequently hypothesized drivers of zoonotic disease emergence and included (Table 1): human presence/activity, animals/hosts, the environment, and reporting effort. Explanatory variables came from a variety of data sources, and all were rescaled or transformed to a spatial grid of 1° resolution (WGS84, c. 110 km at the equator) prior to their use in models. Full details of sources, original resolutions and rescaling are presented in Tables 1 and 2.

Table 2 Original resolutions and extents of source data sets

Source data set	Spatial resolution	Temporal resolution and extent
GRUMP (Global Rural-Urban Mapping Project)[39]	0°5′	5 years, 1970–2000
HYDE (History Database of the Global Environment)[43]	0°5′	10 years, 1900–2000
GMA (Global Mammal Assessment)[12]	300 m	N/A
GLW (Gridded Livestock of the World)[48]	0.05°	N/A
GEnS (Global Environmental Stratification)[53]	0°0′30″	N/A
EarthEnv[55]	0°0′30″	N/A

"Human Activity" data were compiled and eight predictors derived based on the following rationale: (1) Population density likely influences EID risk in two discrete ways. First, as EID events are defined as diseases emerging in the human population, their frequency—before the effects of other predictors—is assumed to be proportional to population density, with the other predictors modifying the per-person risk of EID events. To represent this, we treated human population as a baseline multiplicative factor in our models[37]. Second, population density may affect transmission dynamics such that EID events in areas of denser population may be more likely to produce outbreaks large enough to be detected[38]. We used the Global Rural–Urban Mapping Project[39] human population data set, which provides gridded estimates of human population every five years for 1970–2000. (2) Population change acts as a proxy for changing demands on ecosystems leading to environmental perturbation, which has been hypothesized to drive disease emergence[21]. We created a measure for population change by calculating the inter-decadal difference of human population per grid cell. (3) Land-use type represents largely anthropogenic influence on the landscape (as opposed to 'land cover' below) and has been hypothesized to play a role in disease emergence and spatial distribution[19, 21, 40–42]. We used the HYDE data set which estimates the percentage of land-use types in each grid cell of a global data set every ten years for 1900–2000[43] to derive predictors representing percentage of land used for cropland and percentage used for pasture. We also include the layers for Urban Land and Managed/Cultivated Vegetation from the EarthEnv data set, described below under "Environment", in this category, as they index human impact on the environment. (4) Land-use change has been hypothesized as a key driver for disease emergence by perturbing ecosystems and bringing humans into close proximity with wildlife[5, 7, 8, 21, 27]. We created metrics of change for pasture and cropland by calculating the between-decade difference in values for each grid cell for cropland and pasture.

For data sets with multiple temporal layers (human population, cropland, and pasture), we included the intersection of available dates in different data sets (decades 1970–2000) and calculated inter-decadal change layers by differencing consecutive decades. All presence and absence samples drawn for each event (see below) were matched to the nearest decadal layers (years ending in 5 were rounded up) and the change layer for the decade they fell in.

"Animal/host" data were represented by two predictors: (1) Mammalian biodiversity. The diversity and prevalence in a host population of potentially zoonotic pathogens in an area is hypothesized to be a key factor in the risk of novel pathogen emergence[8, 21, 44]. However, spatial data on global pathogen diversity do not currently exist, and it is estimated that we have identified less than 1% of mammalian viral diversity[36]. Consistent with previous studies, we therefore assume that the number of available pathogens in an area is proportional to the diversity (species richness) of wildlife species[4, 5, 36, 45]. The overwhelming majority of emerging zoonoses have mammalian hosts[46], and global biogeographic patterns of human infectious diseases is highly correlated with global patterns of mammalian diversity[30]. We therefore used mammal biodiversity (species richness), measured as number of mammal species per grid cell as a proxy for pathogen species richness. To do this, we used the most up to date mammal species distribution maps available, derived from species distribution ranges filtered according to species-specific habitat preferences[12]. These habitat suitability models reflected species preferences for land cover types, their altitudinal limits, their tolerance to human presence, and their relationship with water bodies. The full-resolution mammal biodiversity data (representing all 5291 terrestrial mammal species)[12] was rescaled to the study grid by summing the number of species' distributions that overlapped each grid cell; (2) Domestic animal density. A number of past EID events with wildlife origin have emerged through farmed or domestic animal intermediate or amplifier hosts (e.g., Hendra and Nipah virus, SARS). In addition, there is growing evidence that the global trend of intensification of livestock production increases the emergence risk of novel wildlife origin zoonoses, e.g., Nipah virus in Malaysia[47], influenza viruses, and others[6]. We used the Gridded Livestock of the World (GLW) data set[48], which contains data for poultry, goat, buffalo, cattle, sheep, and pig headcounts. We summed mammals to a single predictor (livestock mammal headcount) and retained poultry as a discrete predictor.

We analyzed eight predictors from two data sets representing "Environmental" variables: (1) Climate. Climatic factors have been repeatedly hypothesized as important in the global biogeography of human infectious diseases, including EIDs[30, 49, 50]. Climate may influence disease distribution through enhanced suitability for vectors of wildlife origin zoonoses (e.g., West Nile virus), more rapid vector reproduction rates and biting rates, changes in the efficiency or rates of pathogen transmission among hosts and vectors, and changes in the ability of pathogens to persist in the environment, among other factors[51, 52]. Climate was represented by a single layer in our study, the Global Environmental Stratification[53], which uses a quantitative model to stratify the Earth's surface into zones of similar climate on a single scalar measure, where higher values equate to warmer, wetter (more tropical) regions; (2) Land cover type: Land cover type is associated with the distribution of terrestrial mammals[12] and other taxa[54], potentially exposing humans present to different assemblages of viral species. It is also likely that the types of contact between wildlife and people vary with land cover type. For land cover, we used the EarthEnv data set[55], which divides the Earth's surface into 12 classes. These include different classes of natural ecosystems, urban land and cultivated vegetation (grouped with "Human Activity" above). We excluded barren areas, open water and snow/ice due to a lack of biologically plausible mechanisms for disease emergence. EarthEnv represents each class as a percentage per grid cell.

Reporting effort. The distribution of reported EID events is likely strongly influenced by an inconsistent spatial distribution of detection and reporting of disease outbreaks. Previous studies have used proxies of reporting effort such as the interpolated locations of known sampling sites ("sampling effort")[56]; frequency of countries of residence for all authors of all articles in the Journal of Infectious Disease ("reporting effort")[4]; and PubMed searches for keywords for each country ("reporting bias")[25]. Other studies have used occurrence records for a similar class of observations as a surrogate for background sampling effort; for example, in ecology, modeling the distribution of a particular species and utilizing occurrence records from multiple other species to represent background samples[57].

We adapted these approaches by deriving an index for reporting effort based on the spatial distribution of toponyms (place names) in peer-reviewed biomedical literature. We wrote a Python package, PubCrawler (see Supplementary Methods for full details), to search the full text of each of the 1,266,085 (as of April 2016) articles in the PubMed Central Open-Access Subset (PMCOAS)[58] for toponyms from the GeoNames database[59], which includes data on population (if appropriate), country, and geographical coordinates for each toponym. PubCrawler uses a set of heuristics, based on textual and geographic features of the identified toponyms, to minimize the number of false positives and select amongst ambiguous matches. We selected articles matching terms from the HIuman Disease Ontology[60] and exported extracted toponyms. After excluding a further round of potentially spurious matches, place name matches were assigned a weight, normalized by article, and then summed to the study grid. To impute missing data (resulting in a number of zero-value grid cells) and smooth noise in the raw output, we fit a Poisson boosted regression tree model (using human population, accessibility, urbanized land, DALY rates, health expenditure, and GDP as predictors), and used this to represent reporting effort in our model. This approach produced a layer that adequately represented the underlying data while achieving a similar coverage of grid cells to other layers.

Statistical framework. We used boosted regression trees (BRT) to model EID occurrence[26, 49, 61] and to determine how conditions varied between locations where EID events have been observed compared to areas where they have not. BRTs handle non-linear relationships and higher order interactions among many variables more robustly than many other modeling methods, and are robust to monotonic transformations of data[26, 61]. They fit potentially complex, non-linear relationships by aggregating the predictions of multiple simpler models, and are trained iteratively on random partitions of the data[26, 61]. In addition, predictive accuracy of BRTs, as determined by common validation methodologies (e.g., Area Under the Curve of the Receiver-Operator Characteristic (AUC of the ROC), True Skill Statistic (TSS)), frequently exceeds conventional linear methods[26]. Unlike conventional models, they do not produce confidence intervals or *p*-values.

Resampling regimes. We employed various resampling techniques to incorporate our measure of reporting effort[57, 62], estimate the predictive power of our models, account for spatial uncertainty in EID events[15], and generate empirical confidence intervals for effects representing both sampling uncertainty and spatial

uncertainty[63]. Each time an event was sampled, one presence point and one absence point were drawn (artificially fixing overall prevalence at 0.5)[15]. The presence point was from the grid cells overlapped by that event's polygon, and the absence point from all grid cells; both were weighted by reporting effort (the effect of weighting presence points by reporting effort made little difference for points with small, precisely specified occurrence polygons, and for events with high uncertainty it acted as a prior, specifying that, in the absence of other knowledge, the event was more likely detected where reporting effort was higher).

All replicate BRT models were fit using the R packages dismo and gbm[26]. The function gbm.step() was called with the parameters tree.complexity = 3 (governing interaction depth), learning.rate = 0.0035 (setting the "shrinkage" applied to individual trees), and n.trees = 35 (governing the initial number of trees fit, as well as the "step size" or number added at each step of the stagewise fitting process)[26]. These values were selected through an iterative process, starting with the default parameters, adding tree complexity, and tuning the shrinkage and step size parameters to achieve successful gradient descent consistently across resampling runs, following refs. [26, 63]. With the final parameters, the BRTs composing the bootstrap model fit a mean of 1005 trees.

Our main model used a bootstrap resampling regime, which was used to fit 1000 replicate models. For each model, 147 events were drawn randomly with replacement from the set the 147 EID events of interest, and for each selected event, 1 presence and 1 absence value were drawn as described above. The fitted models were used to generate Relative Influence box plots and Partial Dependence plots with empirical 90% confidence intervals. The mean of the predictions of these models were used to generate all maps.

To compute validation statistics (described below), we conducted 100 rounds of 10-fold cross-validation[15, 63]. In each round, a single presence and absence sample were drawn for each event, which were assigned randomly to ten groups. Each group in turn was held out, and a model was trained on the remaining groups' samples. The model's predictions for the presence and absences samples of the held-out group were used to construct confusion matrices, and calculate the AUC and TSS. This process was repeated 100 times, and the median, 0.05 and 0.95 quantiles for all scores were reported.

Factoring reporting bias out. We assumed that the distribution of observed EID events was conditional on the distribution of reporting effort across the globe following[57]. We fit our main, "weighted" model with grid cells sampled and weighted by reporting effort. The model thus produced a response relative to reporting effort. We multiplied this response by the value of reporting effort in each grid cell to map the index of observed EID event risk (Fig. 3a).

We produced the estimate of the risk index after factoring out reporting bias (Fig. 3b) as follows. We assumed that the optimal distribution of reporting effort for human disease events in a location is proportional to the distribution of the human population. In reality, other unmeasured factors likely affect this. However, given this assumption, we can define reporting bias as proportional to the ratio of reporting effort to the human population (Fig. 4).

$$\text{Reporting bias} \propto \frac{\text{Reporting effort}}{\text{Population}}$$

When bias is known, it is possible to estimate the true distribution of a phenomenon by "factoring bias out"[57]. In ecological studies, this generally means dividing by the measured "survey effort", assuming that the optimal distribution of search effort is uniform across the landscape.

$$\text{True risk index} \propto \frac{\text{Observed risk index}}{\text{Reporting bias}}$$

We posit that, in the case of human disease events, uniform search effort across a landscape is also suboptimal, and that it is safer to assume optimal reporting effort distribution would be proportional to the human population. In this case, we remove "bias" by factoring out measured reporting effort and factoring in assumed optimal effort, and obtain a hypothetical map of the true event risk index, thus:

$$\text{True risk index} \propto \text{Observed risk index} \times \frac{\text{Human population}}{\text{Reporting effort}}$$

Model validation and performance. We used multiple tools for model validation and performance. For our bootstrap model, we calculated deviance explained using the gbm.step() function[26] and also derived median and empirical 90% CIs by taking the 0.05, 0.5, and 0.95 quantiles of those values for the replicate models. Since this model is fit relative to reporting effort, percentage deviance explained is calculated relative to that variable. For the ten-fold cross-validation runs, we calculated the AUC, a threshold-independent measure of model predictive performance that is commonly used as a validation metric in species distribution modelling[64]. The AUC can be interpreted as "the probability that the model will rank a randomly chosen presence site higher than a randomly chosen absence site"[65], or more accurately in our application, a measure of a model's performance to discriminate EID events from random points[57]. Because the use of AUC has been criticized for its lack of sensitivity to absolute predicted probability and its inclusion of a priori untenable prediction thresholds[13], we also calculated the True Skill Statistic (TSS)[15].

Because all test statistics and figures from our main model are relative to the reporting effort measure, we also ran "unweighted" models. We expected these would score yield higher cross-validation scores, since we expected that reporting effort would be correlated both with some important predictor variables and the outcome, and weighting background samples uniformly rather than according to this variable would present a clearer contrast. To avoid bias from land area in the WGS84 grid cells, we additionally weighted our "unweighted models" by land area per grid cell. The figures from these models are presented fully in Supplementary Information.

Code availability. All data and code used to generate the models are available on GitHub (doi: 10.5281/zenodo.400978)[66], as is the code used to generate the reporting effort layer (doi: 10.5281/zenodo.400977)[67].

Data availability. The data sets analyzed during this study are included in this published article and its Supplementary Information Files, with the exception of EID Event shape files, which are available from the corresponding author on reasonable request.

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Author contributions

T.A. and K.M. designed the statistical approach, with contributions from K.J.O. and C.Z.-T. The EID database was updated under P.D.'s supervision. T.A. wrote the modeling code and generated the figures, and N.B. and T.A. wrote the code to generate the publication bias layer. C.R. and M.D.M. contributed the mammal species richness data set. T.A., K.M., K.J.O., and P.D. wrote the manuscript, with all authors contributing edits.

Additional information

Supplementary Information accompanies this paper at doi:10.1038/s41467-017-00923-8.

Competing interests: The authors declare no competing financial interests.

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Query No.	Description	Author's Response
AQ7	References 19 and 41 were duplicates hence reference 41 has been deleted. Please check.	
AQ8	Please confirm the edits to sentence 'We fit our main…' are ok.	
AQ9	Please check and provide complete details for references 66 and 67, if available.	
AQ10	Please provide volume no. and page range/article no. for ref. 34.	
AQ11	Please check ref. 58 is ok.	
AQ12	Please check, figure 4 is not cited in text, so cited after the sentence 'However, given this assumption, we can define reporting.....' is ok.	

From: "Munyua, Penina (CDC/CGH/DGHP)" <ikg2@cdc.gov>
To: David De Pooter <d.depooter@onehealthplatform.com>, Jonna Mazet <jkmazet@ucdavis.edu>, Martyn Jeggo [redacted] Amadou Sall <asall@pasteur.sn>, MARK RWEYEMAMU <mark.rweyemamu@btinternet.com>, "Wang Linfa" <linfa.wang@duke-nus.edu.sg>, "William B. Karesh" <karesh@ecohealthalliance.org>, "Dr. Ottorino Cosivi" <cosivio@paho.org>, "Andrew P. Dobson" <dobber@princeton.edu>, "Barton Behravesh, Casey (CDC/OID/NCEZID)" <dlx9@cdc.gov>, malik <malik@hku.hk>, Baljit Singh <baljit.singh1@ucalgary.ca>, "Gerdts, Volker" <volker.gerdts@usask.ca>, Marietjie Venter <marietjie.venter@up.ac.za>, Lorne Babiuk <lbabiuk@ualberta.ca>, Susan Kutz <skutz@ucalgary.ca>, Patrick Leighton <patrick.a.leighton@umontreal.ca>, "samuel.iverson@canada.ca" <samuel.iverson@canada.ca>, Craig Stephen <cstephen@cwhc-rcsf.ca>
Cc: Ab Osterhaus <Albert.Osterhaus@tiho-hannover.de>, John MacKenzie <J.MacKenzie@curtin.edu.au>, Chris Vanlangendonck <c.vanlangendonck@onehealthplatform.com>
Subject: RE: 5th International One Health Congress: proposing AMR co-chair and keynote speaker
Sent: Tue, 10 Oct 2017 12:58:51 +0000

Apologies, I realized my message was incomplete--Prof Njenga is a Research Professor at Paul Allen School for Global Animal Health, Washington State University, Pullman based in Kenya where he conducts his research work on emerging diseases including zoonosis and involved in building public health systems.

Peninah

From: Munyua, Penina (CDC/CGH/DGHP)
Sent: Tuesday, October 10, 2017 3:42 PM
To: 'David De Pooter' <d.depooter@onehealthplatform.com>; Jonna Mazet <jkmazet@ucdavis.edu>; Martyn Jeggo [redacted] Amadou Sall <asall@pasteur.sn>; MARK RWEYEMAMU <mark.rweyemamu@btinternet.com>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; William B. Karesh <karesh@ecohealthalliance.org>; Dr. Ottorino Cosivi <cosivio@paho.org>; Andrew P. Dobson <dobber@princeton.edu>; Barton Behravesh, Casey (CDC/OID/NCEZID) <dlx9@cdc.gov>; malik <malik@hku.hk>; Baljit Singh <baljit.singh1@ucalgary.ca>; Gerdts, Volker <volker.gerdts@usask.ca>; Marietjie Venter <marietjie.venter@up.ac.za>; Lorne Babiuk <lbabiuk@ualberta.ca>; Susan Kutz <skutz@ucalgary.ca>; Patrick Leighton <patrick.a.leighton@umontreal.ca>; samuel.iverson@canada.ca; Craig Stephen <cstephen@cwhc-rcsf.ca>
Cc: Ab Osterhaus <Albert.Osterhaus@tiho-hannover.de>; John MacKenzie <J.MacKenzie@curtin.edu.au>; Chris Vanlangendonck <c.vanlangendonck@onehealthplatform.com>
Subject: RE: 5th International One Health Congress: proposing AMR co-chair and keynote speaker

Hi David,
I am proposing a co-chair for AMR if that is still needed and a keynote speaker—I didn't see a request for keynote speakers but thought I put this here.

1. **AMR co-chair Sylvia Omulo**-- Since May 2016, Sylvia has been working as a post-doctoral fellow at Washington State University, conducting research on antimicrobial resistance in Kenya. She most recently successfully coordinated a point-prevalence survey (PPS) on antibiotic use – the first in Kenya – within the country's largest referral hospital, and is preparing to roll this out to additional facilities within the country. Sylvia is also coordinating another research study that will investigate how resistant bacteria are acquired and transmitted within the community-healthcare continuum. This work builds on her PhD research which investigated the factors that contribute to the maintenance of antimicrobial-resistant bacteria in a low-sanitation urban informal settlement community in Kenya. She holds a PhD in Immunology and Infectious Diseases from Washington State University – USA (2016).
2. Key note speaker – Prof Kariuki Njenga who is known to a number in this committee has expressed interest in giving a keynote talk in one of the sessions under Science and Policy track. The focus would be on implementing OH with focus to Africa with snippets on translating OH science to policy.

Happy to provide a full bio from Sylvia if needed.
Thanks much,
Peninah

Peninah Munyua, PHD
Epidemiologist and Lead One Health Program
Division of Global Health Protection
Centers for Disease Control and Prevention-Kenya
Cell: +254 710 602 787
ikg2@cdc.gov or Pmunyua@cdc.gov

From: David De Pooter [mailto:d.depooter@onehealthplatform.com]
Sent: Tuesday, October 03, 2017 4:24 PM
To: Jonna Mazet <jkmazet@ucdavis.edu>; Martyn Jeggo <[redacted] Amadou Sall <asall@pasteur.sn>; MARK RWEYEMAMU <mark.rweyemamu@btinternet.com>; Wang Linfa <linfa.wang@duke-nus.edu.sg>; William B. Karesh <karesh@ecohealthalliance.org>; Dr. Ottorino Cosivi <cosivio@paho.org>; Andrew P. Dobson <dobber@princeton.edu>; Barton Behravesh, Casey (CDC/OID/NCEZID) <dlx9@cdc.gov>; malik <malik@hku.hk>; Baljit Singh <baljit.singh1@ucalgary.ca>; Gerdts, Volker <volker.gerdts@usask.ca>; Marietjie Venter <marietjie.venter@up.ac.za>; Munyua, Penina (CDC/CGH/DGHP) <ikg2@cdc.gov>; Lorne Babiuk <lbabiuk@ualberta.ca>; Susan Kutz <skutz@ucalgary.ca>; Patrick Leighton <patrick.a.leighton@umontreal.ca>; samuel.iverson@canada.ca; Craig Stephen <cstephen@cwhc-rcsf.ca>
Cc: Ab Osterhaus <Albert.Osterhaus@tiho-hannover.de>; John MacKenzie <J.MacKenzie@curtin.edu.au>; Chris Vanlangendonck <c.vanlangendonck@onehealthplatform.com>
Subject: 5th International One Health Congress: Scientific Programme Committee telephone conference on October 6th

Dear Scientific Programme Committee members,

Many thanks for responding to last week's invitation to participate in a TC on Friday 6 October 2017. Kindly find the agenda and discussion documents for this teleconference attached to this e-mail. Since not all committee members will be available to participate, I will circulate the outcome of the call to all committee members for a final round of feedback.

The call will start on Friday 6 October at 16:00 CET (10am EDT - 10pm AWST/SGT). To join the call, select the appropriate number from the attached list and enter the participant's code: 47450406#

Kindest regards,

David De Pooter
management
ONE HEALTH PLATFORM
It's all connected
d.depooter@onehealthplatform.com
mobile: +32 479 45 74 46
www.onehealthplatform.com

Sent: Tue, 24 Oct 2017 16:00:52 -0700
Subject: Re: Deep Forest Uganda
From: Jonna Mazet <jkmazet@ucdavis.edu>
To: Peter Daszak <daszak@ecohealthalliance.org>
Cc: Kirsten Gilardi <kvgilardi@ucdavis.edu>, Benard Ssebide [redacted] Carlos Zambrana-Torrelio <zambrana@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Erica Johnson <johnson@ecohealthalliance.org>, Evelyn Luciano <luciano@ecohealthalliance.org>, Mike Cranfield [redacted]

That sounds great and happy to support the continuation & Benard's studies.
J

On Tue, Oct 24, 2017 at 9:57 AM, Peter Daszak <daszak@ecohealthalliance.org> wrote:

Great to hear back Kirsten.

I think we can do this – depending on budget needs. There are some NSF rules that we can't fund Ph.D tuition in foreign countries, but I'll check on these and there are simple work-arounds, e.g. by using NSF funds towards his salary and field costs etc.

Bottom line – it would be great to involve you all and support Benard's work. I'm happy to join a committee if you think that's helpful, also, but bear in mind we might not get the grant, of course!!

We'll continue working on the draft, and get details on potential budgets, forms required etc. over to you by Monday next week.

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

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From: Kirsten Gilardi [mailto:kvgilardi@ucdavis.edu]
Sent: Tuesday, October 24, 2017 10:52 AM
To: Peter Daszak; Jonna Mazet
Cc: Benard Ssebide; Carlos Zambrana-Torrelio; Aleksei Chmura; Erica Johnson; Evelyn Luciano; Mike Cranfield
Subject: Deep Forest Uganda
Importance: High

Hi Peter and Jonna:

Benard and I had a good discussion on this today, and he's definitely interested in participating in Deep Forest research again in Uganda, assuming that the grant could support his enrollment in a PhD program at Makerere University in Kampala — possible?

The upshot here is that he was working closely with Julius Lutwama (UVRI Arbovirology Lab Director and our lab partner for PREDICT Uganda — an exceptional person) on a joint Makerere University-UVRI proposal to the Wellcome Trust for graduate fellowships: the draft proposal outlined five graduate felllowships, of which one was for Benard to conduct EID-related research. Makerere ended up putting forward just two fellowship proposals (one on bioinformatics and the other on vector ecology), so that potential source of support for Benard has dried up (in fact, not even sure the two got funded…).

THAT SAID, Benard still has the mentoring support of Julius L. as well as a potential faculty mentor at Makerere U. who knows about Benard's involvement and experience with PREDICT, and who has been encouraging him to take advantage of the opportunity to work on at least a subset of PREDICT Uganda data for a portion of his PhD. Benard expressed his strong wishes to me today that he might benefit from the guidance of you Jonna or other UCD PREDICT leads (Chris and/or Tracey) on his dissertation work, if he were to delve into PREDICT data…

So if new funding for Deep Forest work in Uganda could support Benard in a PhD program at Makerere, that would be exciting. The in-country partner would need to be UVRI or Makerere (Benard can advise), as he would have to step away from his position at Gorilla Doctors to enroll in a PhD program.

What are next steps?

-Kirsten

Begin forwarded message:

From: Peter Daszak <daszak@ecohealthalliance.org>

Subject: Time Sensitive!! Resurrecting our Coupled Natural-Human Systems proposal to NSF on DEEP FOREST

Date: October 17, 2017 at 12:05:17 PM PDT

To: "Jonna Mazet (jkmazet@ucdavis.edu)" <jkmazet@ucdavis.edu>, "kvgilardi@ucdavis.edu" <kvgilardi@ucdavis.edu>

Cc: Carlos Zambrana-Torrelio <zambrana@ecohealthalliance.org>, Aleksei Chmura <chmura@ecohealthalliance.org>, Erica Johnson <johnson@ecohealthalliance.org>, Evelyn Luciano <luciano@ecohealthalliance.org>

Hi Jonna and Kirsten – a few years ago we submitted a DEEP FOREST proposal to NSF CNH and got semi-decent comments back. We'd like to resurrect it and would like to keep the current focus on DEEP FOREST countries. The plan is to use the DF data from Brazil, Uganda and Malaysia as the basis, but ditch Brazil as a country we're going to continue to work in so that the continued fieldwork will be in Malaysia and Uganda.

The deadline is Nov 21st. Carlos is pulling together the draft and the response to reviewers (reviewer comments attached).

I want to first check with you both that you are interested in doing this – I definitely think it's worth a shot considering the fairly positive reviewers' comments. If so, we'll need to rapidly line up all the paper work, budgets, etc. I've cc'd Carlos and Evelyn who will be able to coordinate.

Hope you'll be part of this and looking forward to getting this grant funded!

Cheers,

Peter

Peter Daszak

President

EcoHealth Alliance

460 West 34th Street – 17th Floor

New York, NY 10001

Tel. +1 212-380-4473

www.ecohealthalliance.org

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that prevent pandemics and promote conservation.

From: [redacted]
To: Dennis Carroll <dcarroll@usaid.gov>, Cara Chrisman <cchrisman@usaid.gov>, Brooke Watson <watson@ecohealthalliance.org>, Peter Daszak <daszak@ecohealthalliance.org>, Nathan Wolfe <nwolfe@metabiota.com>, "Eddy Rubin" <erubin@metabiota.com>, Jonna Mazet <jkmazet@ucdavis.edu>
Subject: Request from Thai group for PMAC 2018
Sent: Wed, 25 Oct 2017 00:04:22 +0000

Hi everyone,

Please see the request below regarding a request through the GVP website. They are interested in attending the GVP session at PMAC 2018.
How would you like to handle this?

Best,
REDACTED

From: gvp-request@ucdavis.edu [mailto:gvp-request@ucdavis.edu] **On Behalf Of** Squarespace
Sent: Friday, October 20, 2017 5:49 AM
To: gvp@ucdavis.edu
Subject: [gvp] Form Submission - New Form - 2018 PMAC

Name: John Crawford

Email Address: john.crawford.mil@afrims.org

Subject: 2018 PMAC

Message: Greetings Fellow Zoonotic Colleagues. I trust you are well, and here is to that being true. I realize the 2018 PMAC is by invitation only and has a limited attendance capacity by design. It would be great if a member from the Armed Forces Institute of Medical Sciences (AFRIMS) here in Bangkok could attend. We are a collection of military medical researchers who share your passion for disease detection, prevention, and treatment. With me as jus one such example, my background is a PhD on high path avian influenza, one post-doc on exotic Newcastle and another on HIV, a DVM with a zoonotic research focus, and military assignments on Lassa fever diagnostics in Sierra Leon, MERS-CoV surveillance in the Middle East, malaria clinical trials in Ghana, and now antimicrobial resistance in Thailand. Thank you kindly for your consideration of AFRIMS for this and future endeavors, and I wish you well. Very respectfully, John Crawford, MAJ, US Army Veterinary Corps, AFRIMS, Bangkok, Thailand

(Sent via *Global Virome Project*)

From:	Damien Joly <djoly@metabiota.com>
To:	William B. Karesh <karesh@ecohealthalliance.org>
CC:	Jonna Mazet <jkmazet@ucdavis.edu>;David John Wolking <djwolking@ucdavis.edu>;Tracey Goldstein <tgoldstein@ucdavis.edu>
Sent:	11/15/2017 2:15:24 PM
Subject:	Re: CBEP RFI for wildlife work in Cambodia, Laos, Vietnam

Got it - thanks Billy!

_____

Damien Joly, PhD
Head, Data Research
Metabiota

Member, American College of Epidemiology
Assoc. Adjunct Professor · Dept. of Ecosystem and Public Health · Faculty of Vet. Med. · U. of Calgary
Information Management Coordinator · Emerging Pandemic Threats - PREDICT program

Unit 7, 1611 Bowen Road, Nanaimo BC V9S 1G5
djoly@metabiota.com · *tel +1 250 616 4961* · *skype* damienjoly
http://www.metabiota.com

From: William B. Karesh
Sent: November 15, 2017 2:11:22 PM
To: Damien Joly
Cc: Jonna Mazet; David John Wolking; Tracey Goldstein
Subject: Re: CBEP RFI for wildlife work in Cambodia, Laos, Vietnam

Just the deadline for responding to the RFI. The RFP still has to come out.

BK

William B. Karesh, D.V.M
Executive Vice President for Health and Policy

EcoHealth Alliance
460 West 34th Street - 17th Floor
New York, NY 10001 USA

+1.212.380.4463 (direct)
+1.212.380.4465 (fax)
www.ecohealthalliance.org

President, OIE Working Group on Wildlife

Co-chair, IUCN Species Survival Commission - Wildlife Health Specialist Group

EPT Partners Liaison, USAID Emerging Pandemic Threats - PREDICT-2 Program

EcoHealth Alliance leads cutting-edge research into the critical connections between human and wildlife health and delicate ecosystems. With this science we develop solutions that promote conservation and prevent pandemics.

On Nov 15, 2017, at 8:42 PM, Damien Joly <djoly@metabiota.com> wrote:

Thanks Billy. It seems the submission date has passed?

Damien Joly, PhD
Head, Data Research
Metabiota

Member, American College of Epidemiology
Assoc. Adjunct Professor · Dept. of Ecosystem and Public Health · Faculty of Vet. Med. · U. of Calgary
Information Management Coordinator · Emerging Pandemic Threats - PREDICT program

Unit 7, 1611 Bowen Road, Nanaimo BC V9S 1G5
djoly@metabiota.com · *tel +1 250 616 4961* · *skype* damienjoly
http://www.metabiota.com

From: William B. Karesh <karesh@ecohealthalliance.org>
Sent: November 15, 2017 11:00:50 AM
To: Jonna Mazet; David John Wolking; Tracey Goldstein; Damien Joly
Subject: CBEP RFI for wildlife work in Cambodia, Laos, Vietnam

From: Brooke Watson <watson@ecohealthalliance.org>
Sent: Thu, 16 Nov 2017 17:44:56 +0000
Subject: Re: Conflict with call today
To: Cara Chrisman <cchrisman@usaid.gov>, Dennis Carroll <dcarroll@usaid.gov>, Eddy Rubin <erubin@metabiota.com>, [redacted] [redacted] Jonna Mazet <jkmazet@ucdavis.edu>, Nathan Wolfe <nwolfe@metabiota.com>, Peter Daszak <daszak@ecohealthalliance.org>

Peter and I are available at 2 or 3 - he has to leave at 4 PM for a board meeting and won't be available then.

Thanks,

Brooke

On Thu, Nov 16, 2017 at 12:26 PM [redacted] wrote:

Hi everyone,

Jonna will be available at 3pm ET today, but not later in the day.

As for me, I will be available for all the times Nathan proposed.

Best,

[redacted]

From: Nathan Wolfe [mailto:nwolfe@metabiota.com]
Sent: Thursday, November 16, 2017 9:16 AM
To: Dennis Carroll <dcarroll@usaid.gov>; Jonna Mazet <jkmazet@ucdavis.edu>; Brooke Watson <watson@ecohealthalliance.org>; Peter Daszak <daszak@ecohealthalliance.org>; Eddy Rubin <erubin@metabiota.com>; [redacted]; Cara Chrisman <cchrisman@usaid.gov>
Subject: Re: Conflict with call today

Hi All

I'm not available at 3pm ET today, but could do 2pm or any time after 4pm ET. I'm also free tomorrow after 2pm ET tomorrow if that's helpful. If 3pm ET today is best I can get an update from Eddy (assuming he can join).

Thanks

Nathan

From: Dennis Carroll <dcarroll@usaid.gov>
Date: Thursday, November 16, 2017 at 8:54 AM
To: Jonna Mazet <jkmazet@ucdavis.edu>, Brooke Watson <watson@ecohealthalliance.org>, Peter Daszak <daszak@ecohealthalliance.org>, Eddy Rubin <erubin@metabiota.com>, Nathan Wolfe <nwolfe@metabiota.com>, [redacted], Cara Chrisman <cchrisman@usaid.gov>
Subject: Conflict with call today

All, I have a meeting with Bill Steiger to discuss funding and funding strategies for GVP at the time of our call today. Can we reschedule for later today - after 3:00 ET?

d

--

Dr. Dennis Carroll

Director, Emerging Threats Program

Bureau for Global Health

U.S. Agency for International Development

Office: 202-712-5009

Mobile: 3 REDACTED

--

Brooke Watson, MSc
Research Scientist

EcoHealth Alliance
460 West 34th Street – 17th floor
New York, NY 10001

1.212.380.4497 (direct)
REDACTED (mobile)
1.212.380.4465 (fax)
www.ecohealthalliance.org

From:	Megan M Doyle <mmdoyle@ucdavis.edu>
To:	predict-surveillance@UCDAVIS.EDU <predict-surveillance@UCDAVIS.EDU>
CC:	Catherine Machalaba <machalaba@ecohealthalliance.org>;William B. Karesh" <karesh@ecohealthalliance.org>;Jonna Mazet <jkmazet@UCDAVIS.EDU>
Sent:	11/27/2017 9:47:17 AM
Subject:	Next surveillance team call this Thurs Nov 30th @ 10am PT/1pm ET

Hi Surveillance Team,
Notes from our last call are attached – please let me know if you have any corrections.

Also, our next call will be this **Thurs, Nov 30th @ 10am PT/1pm ET**. We will follow up with an agenda soon.

Megan

Megan Doyle
Research Associate
Emerging Pandemic Threats PREDICT Project
EpiCenter for Disease Dynamics
One Health Institute
UC Davis School of Veterinary Medicine
530-564-2133
mmdoyle@ucdavis.edu
skype: megan.m.doyle

November 9th, 2017 Surveillance Team Call

Action Items and Reminders for next call:

1. Next meeting Thurs, Nov 30th
2. In the human questionnaire, please ensure teams are checking all livelihoods that apply and filling out all associated modules.
3. To facilitate submission of accurate data to the IM team, each global lead should designate a person on their team who will be responsible for review and QA of data before it is submitted to the EIDITH team (see EIDITH QA guidance for country teams attached)
4. Asia country updates on next call; field and lab activities updates, GHSA highlights, zoonotic disease prioritization workshops, update on data entry and any hurdles/concerns.

Participants: Marcy Uhart, Terra Kelly, Chris Kreuder Johnson, Megan Doyle, David Wolking, Kidan Araya, Jaber Belkirhia, Jennie Lane, Marcy Uhart, Corina Monagin, Brian Bird, Jim Ayukekbong, Matt LeBreton, Tammie O'Rourke, Ashley Lucas, Dan O'Rourke, Emily Hagan, Leilani Francisco, Stephanie Martinez, Emma Lane, Kevin Olival, Saba Qasmieh, Hong-Ying Li, Leti Gutierrez , Patrick, Mindy Rostal, Sarah Olson

Welcome to Jaber Belkirhia from UC Davis who will be supporting PREDICT in Guinea and Senegal!

IRB renewal RB renewal was approved

- The IRB renewal was approved – the approval letter will be uploaded to EIDITH.

Additional testing beyond P2 strategy

- USAID asked us to note where we need to do additional testing beyond what is already funded in our budget for year 4 testing. This is likely a longshot budgeting exercise, but we wanted check in on the plans for year 4 testing with consortium leads and ask what you feel is not yet funded. Based on budgets provided with year 4 workplans, we've reached out to a couple of you already. No other action needed at this time.

Brussels meeting – what would be most useful from the country coordinator perspective?

- Below are some of the ideas discussed on the call – please reach out to Chris & Megan if you have additional ideas for the surveillance section.
    - Outbreak response with respect to lessons learned, managing political sensitivities, and an overall refresher on PREDICT outbreak assistance guidelines;
    - Summaries of global surveillance data presented in NY to help CCs get a better sense of the broader project;
    - Providing timely government reports, reporting to Missions, understanding reporting chain including USAID/Washington;
    - Surveillance challenges and strategies to overcome these challenges; discussion on troubleshooting on the syndromic surveillance side; coordinating human sampling with behavioral work & planning for community engagement;
    - Review of specific protocols– surveillance priorities & rodent sampling strategy Revisit continuing guidance on testing priorities with budget constraints;
    - Breakout session on issues with EIDITH;

Human questionnaire – livelihoods and associated modules

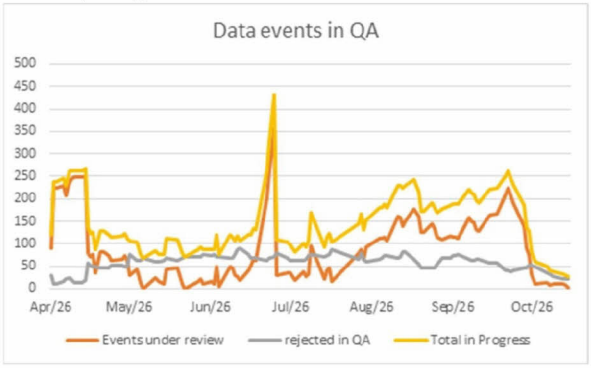
- Guidance going forward for selecting livelihoods/modules in the human questionnaire – **please check all livelihoods that apply to the person being interviewed and fill out all associated modules. We want to be able to capture all the things people we interview are exposed to.** This question was always intended to be "check all that apply," but there was some confusing guidance in the questionnaire for teams who first began using the questionnaire in the very early stages. The guidance has been since updated when we rolled out the human questionnaire more broadly and realized the forms didn't match the guidance. Teams that have been using only the primary livelihood and filled out only one module, please work with IM team to demarcate when your team has begun to use "select all that apply" so you can be sure that when you analyze the data later you can account for this change.

Human specimen labels

- When downloading human questionnaires, IM team has added the ability to also choose to generate specimen labels for the bubble sheets generated. Once you check this box, you will be prompted for the type of specimens you plan to collect and the number of each. Once you click submit, EIDITH will generate 2 files, one PDF containing the bubble forms and 1 Word template which can be used to print your CL-23 cryo labels. The specimen IDs will be coded using the normal EIDITH ID protocol, using the last 6 digits on the barcode as the numerical portion of the ID. The human ID portion of the specimen codes generated (eg: CMAH872624) should be used as the Participant ID when entering the data into EIDITH.

Community engagement

- USAID would like us to track any community meetings, outreach, or sensitization our team conducted during our surveillance events for Monitoring & Evaluation reports. To aid in the reporting, there are 3 new fields to the Site & Event form. The new field is not mandatory and one you click "yes," you will be prompted to enter a date and some notes around what took place during the community engagement session. If you have any questions about how to record this information, please contact your global lead for information.



EIDITH QA status – the number of events under review is now back to a manageable amount. Global leads -- please be sure to identify a person on your team for QA as fixing the most common issues before submission has been helping to speed up the overall QA process (see QA guidance attached to these notes).

EIDITH indicator update

Indicator	Total	NewInLast2Weeks
# countries with data	30	0
# animals sampled	51733	768
# humans sampled	6030	867
# specimens	292370	10355
# tests	155894	3077
# animals tested	16529	
# humans tested	1063	
# animal specimens tested	28434	
# human specimens tested	1434	
# tests active testing ongoing	1309	
# tests waiting interpretation	562	
average days between event and data submission	86	
average days between event and data submission for data submitted in last 2 weeks	43	
number of events/test batches waiting for country input	18	
number of events/test batches waiting for IM review	19	

Africa country updates; field and lab activities updates, GHSA highlights, zoonotic disease prioritization workshops, update on data entry and any hurdles/concerns.

Egypt – Next sampling trip planned for December.
Jordan – Working out an agreement to send human serological samples to Center of Excellence for Influenza Research and Surveillance (CEIRS) in Egypt use their microneutralization assay.
Ethiopia – Continuing bat and primate sampling. FAO now wants to share camel samples and funds for testing. Human syndromic MOU is place and IRB is approved. Back in field next week after some civil unrest.
Kenya – Election shut down occurred early August. Activities resumed and animal (invasive bats, rodents, NHP) and human surveillance took place in Laikipia in late Sept – early Oct. About 2600 samples collected; dry season concurrent sampling is complete. All human data is uploaded to EIDITH; animal data entry on-going. PREDICT offered to do consensus filovirus PCR on recent suspected? Marburg case.
Tanzania –MOH got involved w/3-4 village for Marburg outreach. Get info to share for other countries in the region.
Uganda – subcontract for new laboratory partners (UVRI) approved, will initiate testing bat, rodent, and NHP samples soon. Field and human surveillance activities well underway. Starting discussion on adding a second concurrent sampling site in Y4 in the Queen Elizabeth (National Park) Conservation Area.
Rwanda – actively sampling wildlife. Field Veterinarian is on leave. The National Reference Laboratory, is working on human samples; to address a backlog of wildlife samples that need to be tested, will be sending a large batch of wildlife samples to UC Davis. Shifting to human surveillance at Nyongwe National Park. Y3 animal and human surveillance targets met.
Eastern DRC – The country coordinator has been focusing sampling efforts on baboons, but this got side-tracked this past week by a difficult orphan mountain gorilla clinical case; human surveillance is underway. 150 mountain gorilla fecal samples from the 2016 Virunga-wide census (Rwanda, Uganda and eastern DRC) to UCD, which will be screened for PREDICT priority viral families. Human surveillance underway.
DRC – Sampling bushmeat in Kinshasa including 5 NHPs. For Y4 – 22 human patients sampled so far.

RoC – current focus in RoC is on human behavioral activities in bushmeat markets around Brazzaville; completed 12 human questionnaires and 50 ethnographic interviews in bushmeat markets and are in the process of uploading this data into EIDITH. Planning to conduct a focus group around bushmeat in an island partway between Brazzaville and DRC. Working with MB to compile surveys and start entering into EIDITH.

Cameroon – Animal sampling target complete for Y3 and all data is in EIDITH. Started human surveillance on Sept 25th – ahead of that trained more than 20 hospital staff in preparation for surveillance launch. Enrolled 19 patients for syndromic surveillance, 24 for community. Government asked PREDICT staff to assist in the working groups on Zoonoses, National Laboratory Systems and BSS. USAID PREDICT's contribution to developing pilot wildlife disease surveillance programs was explicitly mentioned by the GoC presenter during the Zoonoses session. Shared die-off events; bats (n=100), one known coronavirus & single gorilla, negative for Ebola) results with the GoC. Dry season sampling end of November.

Guinea – IRB approved for behavioral studies. PCR machine has been repaired so testing should resume soon. Early February -- planning refresher training & starting sampling – still progressing with first sample shipment

Sierra Leone – The teams resumed animal sampling in Sept/Oct during a joint training with team members from Senegal and Guinea. During this training a refresher course on PPE, biosafety, the core basic EIDITH modules, and other lessons learned across all three countries were completed and shared. The training was a success and the teams proceeded to sample bats and rodents for 3 days/nights. A total of 42 bats and 6 rodents were sampled. Restarted field sampling – cave site near Freetown.

Cote d'Ivoire – collected samples from n=152 bats and n=54 rodents. EHA joining upcoming sampling trip and launch event. Met with community leaders, police force, etc., and received very positive response for syndromic surveillance. Began sampling humans in Sept/Oct. 35 samples from humans, workshop on sanitary and security plan meeting…

Liberia – Preparing the next shipment to Columbia for 2500 samples in early Sept. IRB approved for EHP questionnaire in country. All data in EIDITH. Second batch of 1000 samples sent to CU. Lab training in first week of December.

Senegal – Animal team trained in safe sampling, PPE etc. 104 humans enrolled and starting community engagement enrollment in Oct with 150 participants.

Ghana – Aiming to launch clinic work by October. Testing has been initiated.

Next call
Thurs, Nov 30th, 2017