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Faculty of Veterinary Medicine

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Epidemiology of Viral Haemorrhagic Fevers: Seroprevalence and Risk Factor Modelling of Ebola and Marburg viruses in Uganda

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Luke Nyakarahuka

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Department of Food Safety and Infection Biology

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DEDICATION

To

Doreen Asiimwe Buhwa

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LIST OF ACRONYMS AND ABBREVIATIONS

AOR	Adjusted Odds Ratios
CDC	Centers for Disease Control and Prevention
CFR	Case Fatality Rate
CI	Confidence Intervals
DRC	The Democratic Republic of Congo
ELISA	Enzyme-linked Immunosorbent Assay
EPI	Expanded Program for Immunization
ES	Effect Size
EVD	Ebola Virus Disease
FGD	Focused Group Discussion
GIS	Geographical Information Systems
GP	Glycoprotein
GPS	Geographical Positioning System
I^2	Higgin's Statistic for Heterogeneity
MARV	Marburg Virus
MaxEnt	Maximum Entropy
MVD	Marburg Virus Disease
NP	Nucleoprotein
OR	Odds Ratio
PE	Participatory Epidemiology
PPE	Personal Protection Equipment
RAVV	Ravn Virus
RNA	Ribonucleic Acid
VHF	Viral Haemorrhagic Fever
VP40	Viral Matrix Protein
WB	Western Blot
WHO	World Health Organization

SUMMARY

Ebola and Marburg virus diseases, caused by *Ebolavirus* and *Marburgvirus* (filoviruses) respectively, are viral haemorrhagic fevers of public health importance. This is because of associated severity and impact on global health as it happened in Ebola Virus Disease (EVD) outbreak in West Africa in 2014 where the virus spread to other parts of the world. Uganda has reported eight filovirus outbreaks between 2000 and 2016. These outbreaks are thought to be caused by the interaction of people with animals such as non-human primates, bats and other unknown filovirus reservoirs.

In this thesis, a systematic review and meta-analysis approach was used to pool all case fatality rates (CFR), and seroprevalence of EVD and MVD reported up to the year 2015. Occurrence points for filovirus outbreaks in Uganda and selected environmental variables were used in a species distribution model using MaxEnt software to develop risk maps for filovirus outbreaks in Uganda. Further, a questionnaire was administered and focus group discussions conducted to assess risky practices, knowledge, and attitudes toward EVD and MVD outbreaks.

Blood samples were collected from apparently healthy humans and domestic animals (cattle, goat, sheep, pig and dog) from Ibanda, Kamwenge and Luweero districts in Uganda and tested for the presence IgG antibodies against ebolaviruses and Marburg virus using different serological approaches.

The weighted average CFR of EVD estimated from the meta-analysis was 65.0% (95% CI (54.0–76.0%), whereas that of MVD was 53.8% (26.5–80.0%). The overall seroprevalence of Ebola virus from published literature was estimated at 8.0% (5.0%–11.0%), whereas that for Marburg virus was 1.2% (0.5–2.0%). The most severe species of ebolaviruses was *Zaire ebolavirus* while *Bundibugyo ebolavirus* was the least severe. This review showed that EVD and MVD still present with high lethality and low prevalence and their epidemiology still needs to be elucidated.

The filovirus outbreak risk map developed predicted areas that had not reported outbreaks before, including Eastern and North-Eastern parts of Uganda. People were moderately knowledgeable about EVD and MVD, their modes of transmission and clinical symptoms; however, there is still stigma suffered by survivors and their affected families.

The overall filovirus IgG antibody seropositivity in human samples was 2.6% (19/724) of which 2.5% (18/724) was to *Sudan ebolavirus*, 0.1% (1/724) was to *Bundibugyo ebolavirus*, and 0.1% (1/724) was to Marburg virus. One individual had IgG antibody against *Sudan ebolavirus* and *Bundibugyo ebolavirus*. The risk factors for filovirus infection in humans identified included mining (OR=3.4, 95% CI; 1.3-8.5), male sex (3.1, 1.01 - 9.5), going inside mines (3.1, 1.2-8.2), cleaning corpses (3.1, 1.04-9.1) and contact with filovirus suspect cases (3.9, 1.04-14.5). This study shows that there is a possibility of Ebola and Marburg virus disease outbreaks going undetected as some people were found seropositive for filoviruses. It also shows that artisanal gold mining and living near bat-inhabited caves is a risk factor for infection with filoviruses.

From the sampled domestic animal species, goats, pigs, dogs, and sheep from Uganda and goats from Democratic Republic Congo (DRC) and Ivory Coast had detectable IgG antibodies against ebolaviruses. Presence of detectable IgG antibodies against ebolaviruses in domestic animals (goats, pigs, dogs, and sheep) shows a potential of domestic species acting as intermediate transmitters between a filovirus wildlife reservoir and human beings.

It is recommended that increased funding to do more research on filoviruses and other related emerging and re-emerging diseases, in general, to understand the epidemiology of these diseases better hence develop effective control and prevention strategies to avert future epidemics.

SAMMENDRAG

Ebolavirus (EV) og Marburgvirus (MV) er virus innen filovirusfamilien og forsaker alvorlige infeksjoner med hemoragisk feber, Ebolavirussykdom (EVS) og Marburgvirussjukdom (MVS). Sjukdommene store betydning med stor dødelig og en dramatisk påvirkning av global helse ble illustrert under Ebola-utbruddet i Vest-Afrika i 2014 hvor viruset også ble spredt til mange andre deler av verden. Uganda er ett av de landene som har hatt mange utbrudd, med åtte rapporterte filovirusutbrudd mellom 2000 og 2016. Disse utbruddene antas å skyldes samspillet mellom mennesker og dyr som primater, flaggermus og andre ukjente reservoarer av EV og MV.

I denne doktorgraden ble det brukt en systematisk litteraturgjennomgang og en statistisk metaanalyse for å vurdere dødeligheten av EVS og MVS og prevalensen av antistoff mot EV og MV, basert på studier publisert fram til 2015. Forekomst av EVS og MVS i Uganda og utvalgte miljøvariable ble så brukt i en artsdistribusjonsmodell ved hjelp av dataprogrammet MaxEnt for å utvikle risikokart for filovirusutbrudd i Uganda. Videre ble spørreskjema og fokusgruppediskusjoner benyttet i endemisk områder for å vurdere hvordan risikofylt praksis, kunnskap og holdninger påvirkes av utbrudd av EVS og MVS. Blodprøver ble samlet fra tilsynelatende friske mennesker og husdyr (storfe, geit, sau, gris og hund) fra Ibanda, Kamwenge og Luweero-distriktene i Uganda og testet for IgG-antistoffer mot EV og MV ved hjelp av forskjellige serologiske teknikker

Den vektede gjennomsnittlige dødeligheten av EVS fra metaanalysen var 65,0% (95% KI (54,0-76,0%), mens det for MVS var noe lavere, 53,8% (26,5-80,0%). Den samlede seroprevalensen mot EV ble estimert til 8,0% (5,0% -11,0%), mens det for MV var 1,2% (0,5-2,0%). *Zaire ebolavirus* ga høyest dødelighet, mens *Bundibugyo ebolavirus* var den minst alvorlige. Resultaten viste at EVS og MVS fremdeles opptrer med høy dødelighet og lav prevalens og at epidemiologien fremdeles må undersøkes nøye.

Risikokartet viste områder som ikke hadde rapportert utbrudd før som inkluderer østlige og nordøstlige deler av Uganda som risikoområder for utbrudd. Folk hadde moderate kunnskaper om EVS og MVS, hvordan de overføres og kliniske symptomer. Det er fortsatt stigma koblet til EVS og MVS som overlevende og deres berørte familier lider av.

Den totale forekomsten av IgG mot EV og MV hos mennesker var 2,6% (19/724), hvorav 2,5% (18/724) var mot *Sudan ebolavirus*, 0,1% (1/724) mot *Bundibugyo ebolavirus*, og 0,1% (1/724) mot MV. Én person hadde IgG-reaksjon mot både *Sudan ebolavirus* og *Bundibugyo ebolavirus*.

Risikofaktorer som ble identifisert, inkluderte kontakt med gruvedrift (OR = 3,4, 95% CI; 1,3-8,5), å være mann (3.1, 1.01-9.5), gå inn i gruvene (OR= 3.1, 1.2 - 8.2), vask av døde (OR=3.1, 1.04 - 9.1) og kontakt med mistenkte tilfeller av EVS eller MVS (OR=3,9, 1,04 - 14,5). Studien viser at utbrudd av EVS og MVS kan pågå uten at de oppdages, samtidig er det koblet en risiko for viruseksponering til gullgruver, og å bo nær flaggermushuler. Det ble påvist en bedret forståelse i samfunnet av disse sykdommene i Uganda, sannsynligvis på grunn av de gjentatte utbruddene.

Blant husdyr ble påvist IgG antistoffer mot EV hos geiter, griser, hunder og sauer fra Uganda og geiter fra DRC og Elfenbenskysten. Påvisbare antistoffer mot EV hos husdyr som geit, gris, hund og sau viser et potensiale for at disse artene kan ha en viss rolle med overføring av virus mellom et reservoar hos ville dyr og mennesker.

Vi anbefaler en økt finansiering av forskning på filovirus og andre relaterte nye og tilbakekommende sykdommer for å bedre forstå epidemiologien til disse sykdommene, og dermed utvikle bedre kontroll- og forebyggende tiltak for å avverge fremtidige epidemier.

LIST OF PAPERS

PAPER I:
NYAKARAHUKA, L., KANKYA, C., KRONTVEIT, R., MAYER, B., MWIINE, F. N., LUTWAMA, J. J & SKJERVE, E. 2016. How severe and prevalent are Ebola and Marburg viruses? A systematic review and meta-analysis of the case fatality rates and seroprevalence. *BMC Infect Dis,* 16, 708

PAPER II:
NYAKARAHUKA, L., AYEBARE, S., MOSOMTAI, G., KANKYA, C., LUTWAMA, J. J, MWIINE, F. N. & SKJERVE, E. 2017. Ecological Niche Modeling for Filoviruses: A Risk Map for Ebola and Marburg Virus Disease Outbreaks in Uganda. *PLOS Currents Outbreaks*.

PAPER III:
NYAKARAHUKA, L., SKJERVE, E., NABADDA, D., SITALI, D. C., MUMBA, C., MWIINE, F. N., LUTWAMA, J. J., BALINANDI, S., SHOEMAKER, T. & KANKYA, C. 2017. Knowledge and attitude towards Ebola and Marburg virus diseases in Uganda using quantitative and participatory epidemiology techniques. *PLoS Negl Trop Dis,* 11, e0005907.

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NYAKARAHUKA, L., SCHAFER, I., BALINANDI, S., MULEI, S., TUMUSIIME, A., KNUST, B., KYONDO, J., LUTWAMA, J. J., & SHOEMAKER, T. 2017. A Retrospective Cohort Study of Seroprevalence of Ebola and Marburg viruses in humans from two different ecological zones in Uganda (Manuscript).

PAPER V:

NYAKARAHUKA, L., KOEHLER, S., THIESEN, U., KANKYA, C., ODOCH, T., MWIINE, F. N., LUTWAMA, J. J., SKJERVE, E & LEENDERTZ, F. 2017. Seroepidemiological Study of Ebola virus in domestic animals from Africa: Detection of IgG antibodies against ebolaviruses in goats from Uganda, DRC and Ivory Coast (Manuscript).

INTRODUCTION

VIRAL HAEMORRHAGIC FEVERS

Viral haemorrhagic fevers (VHFs) are a group of diseases caused mostly by RNA viruses. They infect both humans and animals (zoonotic) and are clinically characterised by acute onset of high fever and sometimes severe haemorrhagic symptoms especially in the late stages of the disease, hence the name viral haemorrhagic fevers. The classical VHFs, which is the focus of this thesis, are caused by viruses in the genera *Ebolavirus* and *Marburgvirus* in the family *Filoviridae.* Other viral families that cause VHFs include *Bunyaviridae (*Rift Valley fever virus and Crimean-Congo Haemorrhagic Fever virus), *Flaviviridae* (Yellow Fever Virus, Dengue, and Tick-borne encephalitides) and *Arenaviridae* (Lasa virus).

Early clinical manifestations of VHFs include a headache, fever, malaise, anorexia, arthralgia and varying degrees of nausea, vomiting, and diarrhoea, which later progress into external or internal haemorrhages, renal failure, and shock. Patients infected with VHFs exhibit these signs with varying degrees of severity and not all of them develop a classic haemorrhagic syndrome (Singh and Ruzek, 2013, Jahrling et al., 2007).

During the early progression of the VHF infection in humans, they present like another infectious disease in the tropics such as malaria, typhoid, or rickettsial infections. This sometimes presents a diagnostic challenge to the clinicians in establishing a proper early diagnosis, as VHFs are confused with other infections in the tropics hence delaying early detection that would be critical in averting epidemics. Animals do not typically develop symptoms as a result of infection with the VHFs, but non-human primates may develop severe symptoms and die from infection with filoviruses.

Whereas some VHFs are transmitted by vectors such as Yellow fever virus, Crimean-Congo Haemorrhagic Fever virus and Rift Valley Fever virus, some do not have a known vector. Filoviruses, for example, are transmitted by close contact with an infected person's body fluids or close contact with a wildlife reservoir. While viruses that cause VHFs are distributed all over the world, some are restricted by the distribution of their reservoirs. Filoviruses, because of their severity, are considered as Biosafety Level 4 pathogens and have a bioterrorism potential. The following sections and most of the thesis will focus on filoviruses (Ebola and Marburg viruses).

FILOVIRUSES

EBOLA VIRUS DISEASE

BACKGROUND

Ebola Virus Disease (EVD) is a severe, usually fatal disease of zoonotic origin. The aetiology of EVD is linked to five species in the genus *Ebolavirus* in the family *Filoviridae.* The species include *Zaire ebolavirus*, *Sudan ebolavirus*, *Taï Forest ebolavirus, Bundibugyo ebolavirus* and *Reston ebolavirus.* These virus species have different pathogenicity, and have been reported in various parts of the world (Nyakarahuka et al., 2016). *Zaire ebolavirus* was the first to be described in 1976 in Zaire, the current Democratic Republic of Congo (DRC) (Commission, 1978) and was responsible for the biggest outbreak in West Africa in 2014 (Dye and Team, 2015). EVD caused by *Sudan ebolavirus* has mainly been prevalent in South Sudan, and Uganda whereas *Bundibugyo ebolavirus* has been reported in Western Uganda and the neighbouring DRC region of Isiro (Wamala et al., 2010, Kratz et al., 2015). *Taï Forest ebolavirus* was reported in one non-fatal case in West Africa in 1994 (Le Guenno et al., 1995) and no human cases have been reported for *Reston ebolavirus* which was isolated in the USA from monkeys imported from the Philippines (Jahrling et al., 1990).

CLINICAL SYMPTOMS OF EBOLA VIRUS DISEASE

In the early stages of the disease, EVD presents with fever, headache and myalgia, followed by gastrointestinal symptoms such as diarrhoea, vomiting, abdominal pains, and dehydration. If not detected early with timely interventions, the infection will progress to a haemorrhagic phase with bleeding from body orifices, neurological symptoms, and shock that is often fatal. In the West African EVD outbreak, for example, the clinical signs reported were fever (87.1%), fatigue (76.4%), loss of appetite (64.5%), vomiting (67.6%), diarrhea (65.6%), headache (53.4%), and abdominal pain (44.3%). Specific heamorrhagic symptoms were rarely reported. “Unexplained bleeding,” however, was reported in 18.0% of cases(Agusto, 2017). In an outbreak of EVD in Uganda, all laboratory-confirmed cases were febrile. Frequent symptoms were asthenia, loss of appetite, cough, nausea or vomiting and diarrhoea (Mupere et al., 2001). Symptoms do not usually come up during the incubation period, which ranges between 2-21 days. Identification of EVD suspect cases is dependent on the epidemiological link and clinical symptoms. The epidemiological link may include contact with a person who had EVD, but this

may not be possible for index cases. People who have a history of contact with bats or non-human primates or are from endemic areas combined with the above clinical symptoms are high-risk suspects and should be isolated and tested.

EPIDEMIOLOGY OF EBOLA VIRUS DISEASE

Figure 1A shows the distribution of reported EVD cases on the African continent. Most of the reported cases of EVD have occurred in Sub-Saharan Africa. Table 1 shows the cases of EVD that have been reported since Ebola virus was first described in 1976. Apart from the recent outbreak in West Africa where 11,325 deaths were reported, most of the outbreaks have reported deaths lower than 300 in number, occurring in low-income countries in Sub-Saharan Africa. In fact, risk maps have been developed, predicting that most outbreaks are likely to happen in this region, potentially affecting a large population (Pigott et al., 2014, Pigott et al., 2016, Peterson and Samy, 2016). These maps, however, are drawn on a large scale and may not necessarily be very helpful for country-specific surveillance efforts. Developing country-specific risk maps for focused monitoring and hence assist in identifying a reservoir or possible source of infection to index cases.

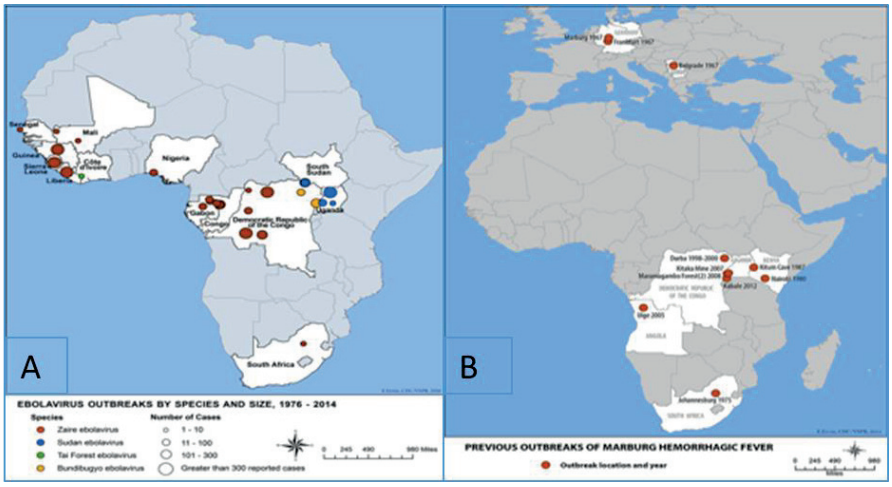


Figure 1: **Map showing the location of filovirus outbreaks: A; Map of Africa showing reported outbreaks of *Ebolavirus* by species. The size of the dot corresponds to the scale of the epidemic. B; Map showing the location of the previous Marburg virus disease outbreaks and year.** (Source: adapted from https://www.cdc.gov/vhf/ebola/outbreaks/history/distribution-map.html)

It seems that the distribution of ebolaviruses corresponds to the allocation of probable wildlife reservoirs. All the four *Ebolavirus* species that cause EVD in humans have been reported in Sub-Saharan Africa. Only *Reston ebolavirus* that is not known to infect humans was detected outside Sub-Saharan Africa-in the Philippines.

Transmission from the natural reservoir (s) yet to be identified may occur when humans get into contact with the reservoir or its body fluids such as faeces, urine, and blood via activities such as hunting and consumption of bush-meat. Because previous outbreaks in Central Africa have been linked to reports of bush-meat consumptions and deaths of wildlife (Leroy et al., 2004a), many hypotheses have been put forward to suggest wildlife such as bats, non-human primates, and antelopes are possible sources of infection.

Table 1: Chronology of outbreaks of Ebola Virus Disease since 1976 to 2014 (Adapted from CDC, https://www.cdc.gov/vhf/ebola/outbreaks/history/distribution-map.html).

Country	Town	Cases	Deaths	Species	Year
DRC	Multiple	66	49	*Zaire ebolavirus*	2014
Multiple countries	Multiple	28652	11325	*Zaire ebolavirus*	2014-2016
Uganda	Luweero	6	3	*Sudan ebolavirus*	2012
DRC	Isiro	36	13	*Bundibugyo ebolavirus*	2012
Uganda	Kibaale	11	4	*Sudan ebolavirus*	2012
Uganda	Luweero	1	1	*Sudan ebolavirus*	2011
DRC	Luebo	32	15	*Zaire ebolavirus*	2008
Uganda	Bundibugyo	149	37	*Bundibugyo ebolavirus*	2007
DRC	Luebo	264	187	*Zaire ebolavirus*	2007
South Sudan	Yambio	17	7	*Zaire ebolavirus*	2004
The Republic of Congo	Mbomo	35	29	*Zaire ebolavirus*	2003
The Republic of Congo	Mbomo	143	128	*Zaire ebolavirus*	2002
The Republic of Congo	Not specified	57	43	*Zaire ebolavirus*	2001
Gabon	Libreville	65	53	*Zaire ebolavirus*	2001
Uganda	Gulu	425	224	*Sudan ebolavirus*	2000
South Africa	Johannesburg	2	1	*Zaire ebolavirus*	1996
Gabon	Booue	60	45	*Zaire ebolavirus*	1996
Gabon	Mayibout	37	21	*Zaire ebolavirus*	1996
DRC	Kikwit	315	250	*Zaire ebolavirus*	1995
Côte d'Ivoire	Taï Forest	1	0	*Taï Forest virus*	1994
Gabon	Mekouka	52	31	*Zaire ebolavirus*	1994
South Sudan	Nzara	34	22	*Sudan ebolavirus*	1979
DRC	Tandala	1	1	*Zaire ebolavirus*	1977
South Sudan	Nzara	284	151	*Sudan ebolavirus*	1976
DRC	Yambuku	318	280	*Zaire ebolavirus*	1976

Figure 2 proposes mechanisms by which EVD spills over from wildlife to the human population. The debate on bats as potential reservoirs of ebolaviruses is inconclusive, as no ebolaviruses have been isolated from bats despite finding some bats seropositive for Ebola virus and others with viral RNA (Leroy et al., 2005). As bats may not necessarily be the primary reservoirs of ebolaviruses, there is a need to look into other (Leendertz, 2016, Leendertz et al., 2016). The role of non-human primates as reservoirs have been unconvincing, as these die from infection with filoviruses (Jaax et al., 1996, Geisbert et al., 2002, Formenty et al., 1999, Rouquet et al., 2005). Other wildlife that has been reported to be infected by Ebola virus was one duiker, whose bone tested positive by Polymerase Chain Reaction (PCR) in the Republic of Congo bordering Gabon (Rouquet et al., 2005).

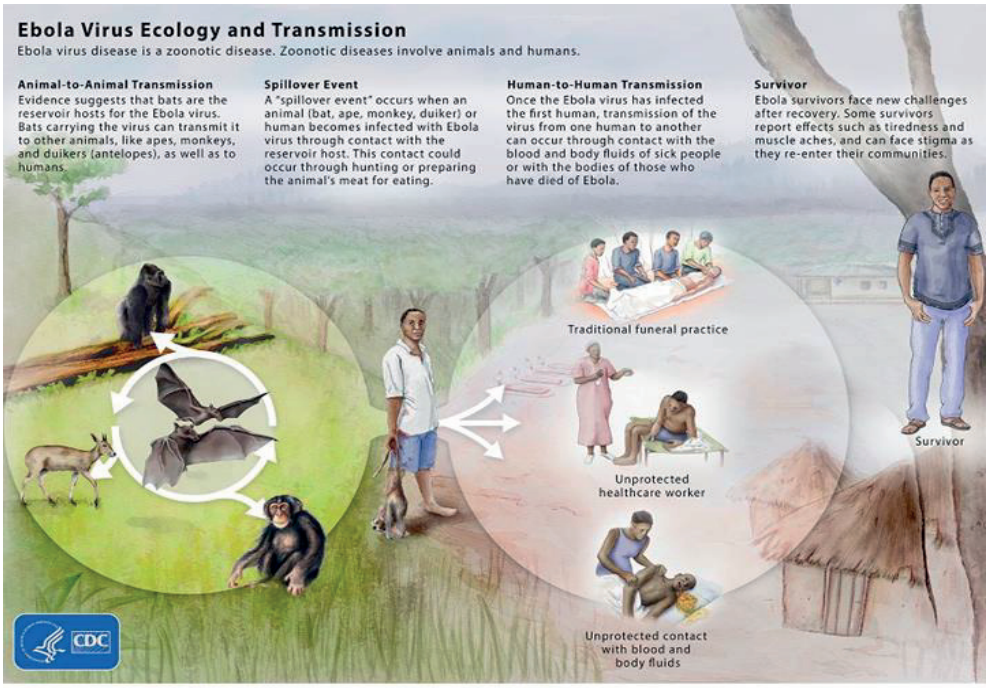


Figure 2**: Proposed Ebola virus ecology and transmission** (Source: CDC, https://www.cdc.gov/vhf/ebola/resources/virus-ecology.html)

Dogs and pigs are the only domestic animals that have been associated with ebolaviruses. Dogs were found to be IgG antibody seropositive in Gabon (Allela et al., 2005) whereas *Reston ebolavirus* has been reported in pigs and they have shown potential for infection with ebolaviruses (Weingartl et al., 2012, Team, 2009, Marsh et al., 2011). However, pigs and dogs

still need to be studied further if they are to qualify as reservoirs for ebolaviruses. A lack of a clear reservoir for ebolaviruses and real source of infection or spill-overs into human populations has been one of the drivers of this research effort.

Once a spill-over event into the human population has occurred, transmission occurs through contact with an infected person, mainly from their body fluids or contact with a corpse, in particular through the practice of funeral rites. Asymptomatic infections in humans that have been assessed through serosurveys could be another exciting turn into the epidemiology of filoviruses, as one would expect infected persons to show severe symptoms.

Use of personal protective equipment is necessary to control transmission, primarily by health care workers and those taking part in funerals. With good health care and symptomatic management, infected individuals can recover from EVD and be re-integrated into the community, but these patients usually suffer from stigma and other sequelae resulting from infection.

MARBURG VIRUS DISEASE

Marburg virus disease (MVD) is caused by Marburg virus that belongs to the same family as *Ebolavirus*, *Filoviridae* and has similar characteristics as EVD. MVD is a severe illness in humans and non-human primates, characterised by haemorrhagic signs indistinguishable from those of EVD. The etiological agent of MVD is Marburg virus (MARV) and the closely related Ravn virus (RAVV). The disease was first described in 1967 in the German city of Marburg when monkeys imported from Uganda infected laboratory workers (Siegert et al., 1968). The incubation period ranges from 2-21 days, depending on several factors. Early symptoms of infection with MVD include sudden fever, fatigue, headache, nausea and vomiting, diarrhoea, rash and conjunctivitis among other signs. As the disease progresses, heamorrhagic signs set in accompanied by multiple organ failures and disseminated intravascular coagulation (DIC). In a multidistrict MVD outbreak in 2012 in Uganda, nearly all confirmed and probable cases (96%) had fever, anorexia, fatigue, headache and vomiting. Half of confirmed and probable case-patients (50%) had haemorrhagic symptoms (Knust et al., 2015). However, MVD, like other related VHFs tend to show non-specific symptoms, and relying on symptoms alone for diagnosis is not enough without laboratory confirmation. This was seen in an outbreak of isolated incident of MVD in Kampala Uganda where the patient was co-infected with malaria (Nyakarahuka et al., 2017).

EPIDEMIOLOGY AND ECOLOGY OF MARBURG VIRUS DISEASE

The epidemiology of MVD is similar to that of EVD, especially regarding distribution and host species. Figure1B and Table 2 show the distribution and the number of outbreaks of MVD that have been reported since 1967-2014. Fewer outbreaks of MVD than EVD have been reported worldwide. Twelve outbreaks of MVD have been reported, 6 of which have either been in Uganda or have been linked to Uganda. The most prominent outbreak happened in the Uige province in Angola, where 252 cases were recorded, with a case fatality rate of 90% (Towner et al., 2006). This was followed in size by the outbreak in DRC Durba where 154 cases were reported (Colebunders et al., 2007). Just like EVD, all outbreaks have been reported from Sub-Saharan Africa with a few cases exported to Europe and USA (Figure 1B).

Unlike EVD, progress has been seen in the search for the reservoirs of Marburg virus. Bats of species *Rousettus aegyptiacus,* caught in the Kitaka gold mine and a Python cave from the Albertine region in Western Uganda have been described as potential reservoirs of Marburg virus (Towner et al., 2009, Amman et al., 2012, Amman et al., 2014, Jones et al., 2015, Amman et al., 2015b). Towner *et al.* (2009), estimated that about 5,000 bats could be infected in a cave inhabited by 100,000 bats. The prevalence was 5.1% (31/611), indicating that these bats could have been the source of the infection for human beings (Towner et al., 2009). This followed an outbreak of MVD in 4 miners who were working in that same cave in 2007 (Adjemian et al., 2011). About 50 km west of the Kitaka mine, is the *Python cave* in the neighbouring district of Rubirizi. Python cave is found inside Queen Elizabeth National Park in Maramagambo forest. It is estimated that 40,000 *Rousettus aegyptiacus* fruit bats are the only species that inhabit this cave. Following infection of Dutch and American tourists in 2007 and 2008 by Marburg virus after visiting this cave (Centers for Disease and Prevention, 2009, Timen et al., 2009), investigations found out the bats in this cave were infected with Marburg virus and 2.5 % of these bats were positive by Q-RT-PCR test (Amman et al., 2012, Amman et al., 2014). The bats in these caves have been linked to three MVD outbreaks, but do not die or develop clinical symptoms from infection with Marburg virus (Amman et al., 2015a, Amman et al., 2015b, Schuh et al., 2017). Transmission of Marburg virus in human populations, just like ebolaviruses happens after a spillover event from the natural reservoir.

Table 2: Reported outbreak of Marburg virus disease between 1967 to 2014

Country	Number of Cases	Number of Deaths	Year & month of outbreak
Germany and Yugoslavia	31	7	1967, August
Johannesburg, South Africa	3	1	1975, February
Kenya	2	1	1980, January
Kenya	1	1	1987, August
Russia	1	1	1990
DRC	154	128	1998, October
Angola	252	227	2004, October
Uganda	4	1	2007, June
The USA from Uganda	1	0	2008, January
Netherlands from Uganda	1	1	2008, July
Uganda	15	4	2012, October
Uganda	1	1	2014, October

SOCIO-ECONOMIC AND CULTURAL IMPACT OF VIRAL HEAMORRHAGIC FEVERS

Outbreaks of VHFs, and especially EVD and MVD have adverse effects economically, socially, and culturally. The impact arises directly from mortality and morbidity, followed by other indirect effects, mainly due to perceived high mortality and morbidity. The fear of being potentially infected with ebolaviruses and die from them is the most obvious one which partly is a natural human response.

This fear, which has been reported in many studies (Kinsman, 2012, Ogoina, 2016, Parmet and Sinha, 2017), including the one presented in this thesis, sets off a cascade of events. These events include fleeing from the affected village, the stigma of the affected, practice of witchcraft and sorcery to avert any imminent death and irrational decision making by the affected communities that ultimately impact on their lives negatively. However, fear and stigma can be positive in a sense that it is accompanied by isolation which is critical in prevention and control measures. Accompanying this fear, the affected communities mistrust the international biomedical teams. They always feel exploited or ignored by these teams instead of making them part of the outbreak response. There is usually a lack of empathy by these teams, and they continuously draw blood from patients without proper feedback which generates negative attitude and despair. These teams are also always competing among

themselves which also contributes to negative feedback from the affected communities (Thiam et al., 2015, Abramowitz et al., 2015, Harman, 2014).

Common in Africa, when a person dies, is the practice of conducting funeral rites. Some cultures must prepare the body in a certain way before it is buried and if this is not done, there is always a belief that the dead person will come back in real life to haunt them. However, during outbreaks of EVD or MVD, this is not respected, which negatively affects the concerned communities (Ravi and Gauldin, 2014). The major and direct impact on EVD disease, however, is the death of people. The impact of a family losing their loved ones and leaving orphans is everlasting, especially for diseases that can wipe out the whole family. Affected families suffer from stigma, as they are sometimes considered 'vectors' for EVD or MVD, and often cannot live a normal life in their communities (Davtyan et al., 2014, Chan, 2015).

Economic impacts come as a result of the loss of revenue, partly because businesses are hampered by the outbreak because people die, and sometimes movement of goods and services is restricted followed by closure of markets and other public gatherings to curtail the epidemic. There is also much alarmism that scares away investors, and business investment is stopped by governments when resources are focused on outbreak control, as it was in affected West African countries in the 2014 EVD outbreak. The overall labour supply and productivity go down, even for expatriates, as many leave the country, and global travel becomes a problem as airlines no longer want to fly to affected countries. This leads to weakening of the tourist sector that helps most of the countries in the tropics to earn foreign exchange.

Because of the stigma associated with the disease, people do not go to hospitals, also increasing the mortality and morbidity of other infectious diseases such as Malaria, dengue, and yellow fever. Vaccinations are stopped, and this can lead to a long-lasting adverse health effect. Another significant impact on the medical care is the death of health care workers in countries where the doctor to patient ratio is even lower. Most of the remaining health workers also run away for fear of contracting the disease and inadequate protective equipment further complicating the situation.

Education services are affected because of the communicable nature of the illness; the schools close during EVD/MVD outbreaks. Bringing back the pupils after the epidemic is costly, but also some students do not come back to school hence long-term effect.

CONTROL AND PREVENTION OF VIRAL HAEMORRHAGIC FEVERS

The control and prevention of VHFs such EVD and MVD depend on the virus transmission dynamics, as well as the public health infrastructure available in affected countries. Outbreaks are believed to occur when a spill-over event happens from a hypothesised animal reservoir in Central African forests to susceptible human populations. How this spill-over event happens is not well understood. Many hypotheses have been put forward, which include hunting and eating of wildlife meat such as bats, antelopes and non-human primates. Understanding how spill-over event occur would be beneficial in averting epidemics.

For MVD, it has been shown that the virus is shed in the saliva of bats (Amman et al., 2015b, Schuh et al., 2017), and since these are fruit bats, the virus can be left on fruits as mangoes, guavas, pawpaws and ripe bananas that are also a delicacy to humans. Eating such a fruit without washing it would start a spill-over event of MVD into the population. With this knowledge, therefore, fundamental hygiene such as washing fruits before eating, boiling water before domestic use could be crucial in stopping spill-overs. Also for MVD, it has been shown that outbreaks are associated with artisanal mining activities where miners invade bats in caves looking for minerals. This usually leads to substantial contact with faeces or saliva of bats that could lead to an infection. If these artisan miners can wear adequate protective equipment, this would be very helpful is stopping spillovers. It is still difficult to stop spill-overs of EVD since we do not know precisely the real reservoirs of ebolaviruses. This was one of the overarching aims of this thesis, hence studying domestic animals was critical.

Once a spill-over of a filovirus into a naïve human population has happened, control measures can be instituted by isolating the infected persons and giving them symptomatic treatment. Listing the contacts of confirmed cases and following them for 21 days, so that once they start showing symptoms of a VHF infection, they are quickly isolated from the general populations. However, these efforts are hampered by poor public health infrastructure in developing countries. Control of EVD depends on early detection and immediate response, which usually delays exacerbating the spread of the disease.

There has been progress in vaccine development for Ebola virus. The vaccine, called rVSV-ZEBOV, was investigated in a trial involving 11,841 people in Guinea during 2015. In the Guinea ring vaccination, an open-label, randomised cluster trial, a vaccine efficacy of 100%

was reported (Henao-Restrepo et al., 2017). Among 5837 people who received the vaccine, no EVD cases were recorded ten days or more after vaccination. In comparison, there were 23 cases 10 days or more after vaccination among those who did not receive the vaccine as reported by the WHO. This vaccine is undergoing approval from WHO and other government agencies, and is available to be used in case of emergencies. Other vaccine trials before the Guinea trial, were not as successful (Trad et al., 2017a). Other possibilities that have been considered for management of filoviruses is the use of antibody-based therapies such as the use of convalescent patient plasma given to acutely ill patients (Van Griensven et al., 2016). Evaluations of the potential drugs and small molecules for the treatment of filoviruses has been on-going and heightened during the West Africa EVD outbreak (Trad et al., 2017b).

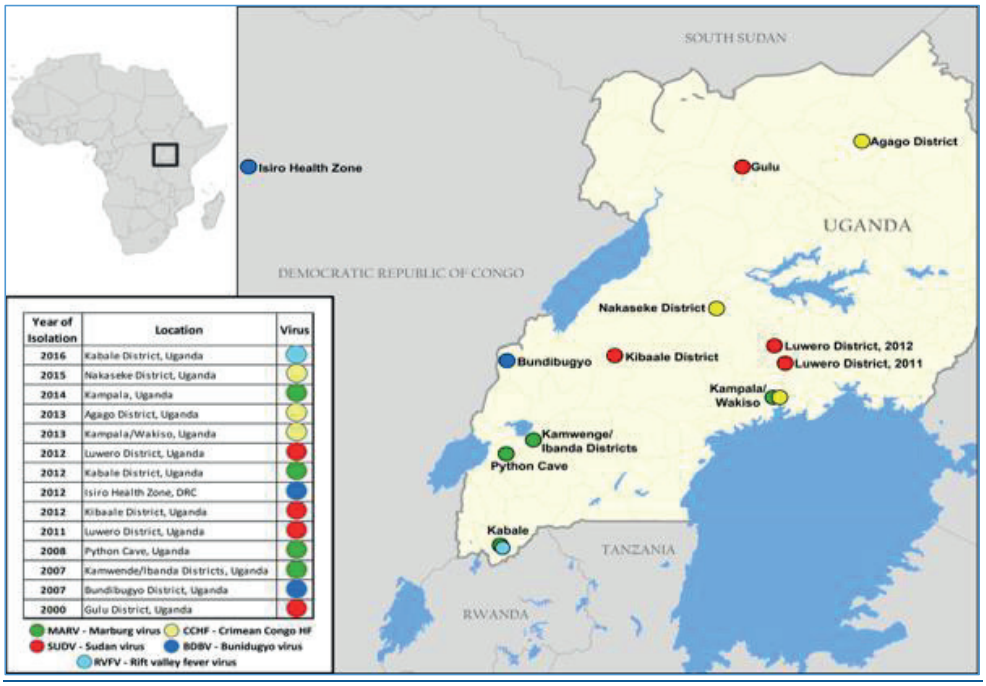
HISTORY OF EBOLA AND MARBURG VIRUS OUTBREAKS IN UGANDA

Uganda has reported more outbreaks of VHFs than other countries in Sub-Saharan Africa (Figure 3). These include five (5) EVD outbreaks and three (3) MVD outbreaks. The first EVD outbreak in Uganda (that remained the most significant EVD outbreak ever recorded in Uganda) occurred in 2000 in the districts of Gulu, Masindi and Mbarara in which 425 cases with 224 deaths (CFR 53%) were reported (Okware et al., 2002). Since then, four additional outbreaks have occurred, including the one in Bundibugyo district in 2007 (147 cases, 37 deaths) (Wamala et al., 2010), in Luweero district in 2011 (1 case, 1 death) (Shoemaker et al., 2012), in Kibaale district in 2012 (24 cases, 17 deaths) and in Luweero district in 2012 (7 cases and 4 deaths) (Albarino et al., 2013). The outbreak in Bundibugyo district was associated with a new strain of *Ebolavirus*, later named *Bundibugyo ebolavirus* (Towner et al., 2008). This strain subsequently caused an outbreak in Isiro, Haut Uélé district, Province Orientale, DRC in 2012 (72 cases and 31 deaths) (Kratz et al., 2015).

Three MVD outbreaks have been recorded in Uganda. The first recorded MVD outbreak was in 2007, where three cases and one death were reported (Adjemian et al., 2011) In a 2012 outbreak, the total count of confirmed and probable MVD cases was 26, of which 15 (58%) were fatal (Knust et al., 2015). The outbreak in 2012 started in Ibanda district, and subsequently spread to at least three other districts. In 2014, Uganda reported only one case that was diagnosed with Marburg virus. This case was co-infected with malaria, for which it was previously treated (Nyakarahuka et al., 2017). Given the fact that no other person to person

transmissions were reported, there are chances of filovirus infections going unnoticed hence the need for active surveillance.

These outbreaks cause loss of human lives and associated morbidities. All these add stress to the healthcare system. Efforts to respond to these outbreaks need lots of resources regarding funds, laboratory testing, and personnel. Usually, when these outbreaks occur, health workers run away from health facilities leaving other patients with no health care.



Year of Isolation	Location	Virus
2016	Kabale District, Uganda	
2015	Nakaseke District, Uganda	
2014	Kampala, Uganda	
2013	Agago District, Uganda	
2013	Kampala/Wakiso, Uganda	
2012	Luwero District, Uganda	
2012	Kabale District, Uganda	
2012	Isiro Health Zone, DRC	
2012	Kibaale District, Uganda	
2011	Luwero District, Uganda	
2008	Python Cave, Uganda	
2007	Kamwende/Ibanda Districts, Uganda	
2007	Bundibugyo District, Uganda	
2000	Gulu District, Uganda	

Figure 3**: Map of Uganda showing locations of Viral Haemorrhagic Fever Outbreaks (With permission from CDC/Uganda Virus Research Institute Viral Haemorrhagic Fever Surveillance Program)**

KNOWLEDGE GAPS

Filoviruses cause highly contagious diseases (Marburg and Ebola virus diseases) characterised by devastating epidemics in recent times. More than 10,000 people died of EVD in West Africa

in 2014, and many more got infected (CDC, 2016). Apart from causing morbidity and mortality, outbreaks of filoviruses cause panic in public, interfere with global travel and have a severe socioeconomic impact as highlighted in the introduction chapter of this thesis. However, there are still many knowledge gaps as far as filovirus research is concerned.

For example, we do not know how the Ugandan communities respond to these outbreaks, what are their attitudes, practices and knowledge on these filovirus outbreaks. There is a need to investigate further, for example, the reported witchcraft that is usually associated with filovirus outbreaks as well as social disharmony and conflict (de Vries et al., 2016). Understanding how communities respond to these outbreaks will give Uganda better entry points during outbreak response.

Ecological niche modeling has been used to study geographical distribution of filoviruses, but mostly it has been on a continental level spanning the Afro-Tropics (Peterson et al., 2006, Peterson et al., 2004, Pigott et al., 2014). We do not know the relationship between climatic zones of Uganda regarding rainfall distribution and temperature, and how these determine the distribution of filoviruses. There is a lack of country-specific risk maps that are accurate and relevant to the country's filovirus surveillance activities. In Uganda for example, there is little knowledge regarding where these outbreaks are likely to occur, when and how they are likely to spread and the number of people they are likely to affect. This information is crucial for preparedness and response in case of outbreaks. We do not have country-specific prediction models showing hotspots for potential filovirus outbreaks. There is a need to do ecological mapping of filovirus hot spots in Uganda in comparison with bat colonies and other probable reservoirs.

Although these filoviruses can be associated with high case fatality rates (Nyakarahuka et al., 2016), many people have recovered from these VHFs. Evidence of antibodies for Ebola and Marburg viruses has been found in apparently healthy individuals (Becker et al., 1992, Becquart et al., 2010b, Gonzalez et al., 2000) in Gabon and the Central African Republic. This would indicate that some people get infected with Ebola or Marburg viruses and recover without the notice of the health care system or the infections are asymptomatic. In a country like Uganda, that has reported the highest number of filovirus outbreaks; we do not know whether we have outbreaks that go undetected or subclinical infections with filoviruses. Hence, there is a need for seroepidemiological studies that will give added information on the

prevalence of Ebola and Marburg viruses, and explain the risk factors. We do not know for example, how Marburg virus outbreaks are related to mining activities, caves, and tourism. Testing of people around caves with bats that have been hypothesised to be reservoirs for these haemorrhagic fevers will help us understand the associated risk factors.

In the 2014 West African outbreak of EVD, investigations could not link the outbreak to any zoonotic origin, although evidence of infection of bat species with Ebola virus was found (Mari Saez et al., 2014). Research has been done on the ecology of filoviruses, but it is not yet substantial. Recent developments involve the discovery of Marburg virus in cave-dwelling fruit-eating bats of species *Rousettus aegyptiacus* in Kitaka and python caves in Uganda (Towner et al., 2009, Amman et al., 2012). Filovirus isolation from other bat species has not been successful, as was attempted in a MVD outbreak in Congo in 1999 (Swanepoel et al., 2007). The role of non-human primates as natural reservoirs is still questionable, as they seem to succumb to filovirus infection (Wittmann et al., 2007, Nidom et al., 2012, Leroy et al., 2004b). Dogs have been found seropositive in Gabon with a seroprevalence of up to 40% (Allela et al., 2005). Serological evidence of filoviruses has also been found in pigs in the Philippines (Sayama et al., 2012), but no isolation of the virus has been possible. Despite the closeness of livestock such as goats, sheep, and cattle to humans in East and Central Africa, their role in the transmission of filoviruses has not been thoroughly investigated. We do not know whether goats in Uganda, are exposed to filoviruses just like their close relative, the duiker that was found positive for Ebola virus (Rouquet et al., 2005), hence playing a role in the spill-over events of filoviruses. There is need to investigate the role of livestock (cattle, goats, sheep and pigs) and dogs in the transmission of filoviruses in an epidemic-prone country like Uganda.

AIMS AND OBJECTIVES OF THE THESIS

The overall aim of this thesis was to describe the epidemiology of filoviruses in Uganda with the primary goal of contributing to knowledge that can help in epidemic preparedness, surveillance for filoviruses and control and prevention in case an epidemic occurs. Specifically, the objectives were as follows.

1. Update the information on prevalence and case-fatality rates of Ebola and Marburg viruses from published literature.
2. Develop ecological risk maps for filovirus outbreaks in Uganda.
3. Describe knowledge and attitudes towards filovirus outbreaks in Uganda.
4. Estimate the seroprevalence and identify risk factors for filovirus infection in humans from selected areas in Uganda.
5. Determine the seroprevalence of filoviruses in domestic animal species and explain risk factors for filovirus infection in selected areas in Uganda.

MATERIALS AND METHODS

Figure 4 shows the five stages of the research process that was followed to generate this thesis. The process started with a systematic review and meta-analysis, then developed risk maps for filovirus outbreaks in Uganda, followed by a knowledge and attitude study and finally seroepidemiological studies in humans and animals. The methods used in each of the research stages are summarised in paragraphs that follow.

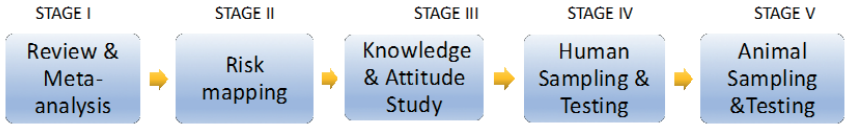


Figure 4**: Research Process**

SYSTEMATIC REVIEW AND META-ANALYSIS (PAPER I)

Published literature on CFR and seroprevalence of filoviruses was retrieved through a search of online databases. Articles were included if they reported deaths, cases, and seropositivity. Information was further cross-referenced with websites of ministries of health of affected countries, WHO, and CDC databases. The effect size was case fatality rate (CFR) and seroprevalence of Ebola and Marburg virus diseases. The analysis was done using the *metaprop* command in Stata (Stata/ SE for Windows, StataCorp, College Station, TX) (Nyaga et al., 2014). The effect size was estimated by use of the random effects model due to observed study differences and presented in forest plots. Heterogeneity was assessed using Cochrane's Q test and the Higgins statistic (I^2). Publication bias was assessed using funnel plots and Begg's bias test. A meta-regression procedure was done to assess if factors such as species, country, year and month of outbreak influence CFR of both EVD and MVD using the traditional logit-transformation.

ECOLOGICAL NICHE MODELLING (PAPER II)

The Maximum Entropy model building software (MaxEnt), a machine learning modelling approach that uses presence-only data was used to establish filovirus – environmental relationships(Phillips et al., 2006). Presence-only data for filovirus outbreaks for Uganda were collected from the field using a GPS receiver mapping households within villages that had

confirmed cases of either EVD or MVD. Additional presence-only data for filoviruses was obtained from online sources (Mylne et al., 2014). Occurrence points for bats in Uganda, the hypothesised reservoirs of filoviruses, were obtained from the online database and from the field.

Environmental covariates (rainfall and temperature variables) from *Africlim* that have been downscaled to a nominal resolution of 1km x 1km for Africa (Platts et al., 2015) were used after testing for collinearity. Presence only coordinates and environmental covariates were later imported into MaxEnt software and model run at default settings. The output of the model was a logistic format prediction map showing the relative probability of the presence of filoviruses survival on a scale ranging between 0 and one obtained from an average of 100 bootstrap runs. Model evaluation was carried out using Receiver Operating Characteristic (ROC) plots and a Jackknife test. Risk maps were developed using ArcGIS 10.3 mapping software.

PAPER III, PAPER IV, AND PAPER V

STUDY SITES

This study was carried out in two different ecological zones in Uganda, one in western Uganda districts of Ibanda and Kamwenge and the other in the central district of Luweero (Figure 5). In western Uganda, sampling was done in the areas where bats of species *Rousettus aegyptiacus* were found to be positive for Marburg virus. These included Ibanda and Kamwenge districts in the Albertine Rift which is part of the Great Rift Valley. This region has a high biodiversity, and has two caves, the Kitaka cave and Python caves that are inhabited by Marburg virus-infected bats (*Rousettus aegyptiacus*). People and animals from this region were considered as high-risk groups, or exposed groups to filovirus infection and especially Marburg virus. These groups were compared with populations in Central Uganda, Luweero district that have experienced two EVD outbreaks and the area is not known to have caves inhabited by *Rousettus aegyptiacus* bats. The paragraphs below briefly explain the study sites.

1. **Kamwenge and Ibanda districts**. The studied areas were around Kitaka bat cave within Kasyoho-Kitomi forest reserve (Figure 5). This is a vibrant ecosystem, bordering Queen Elizabeth National Park, with several caves inhabited by bats. The study focused on human and livestock populations in and around Kitaka cave in this forest reserve since it is known to harbour *Rousettus aegyptiacus* bats that are reservoirs for Marburg virus and may also

have other reservoirs for filoviruses. Blood samples were collected from both humans and domestic animals in and around this forest reserves (Figure 6A).

2. **Luweero district**. The district headquarters of Luweero is located approximately 75 kilometres north of Uganda's capital Kampala. Agriculture is the mainstay of the district economy. It is estimated that 85% of the district population is engaged in agriculture involving both crop and animal husbandry. Livestock is common in the northern areas of Luweero district. It is not known to have caves inhabited by *Rousettus aegyptiacus* and has a different topography and landscape compared to western Uganda, which is mainly grassland savannah (Figure 6B).

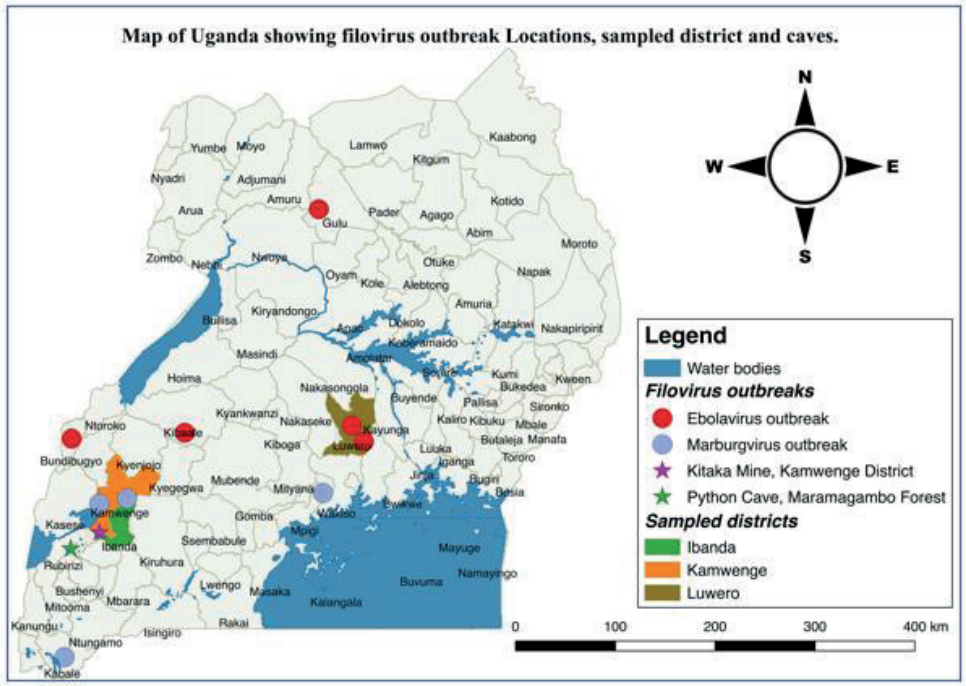


Figure 5: Showing studied districts, reported filovirus outbreaks and location of bat occupied caves in Uganda.

Figure 6: Landscape and vegetation cover of the study sites. **A:** Study site in Western Uganda, Kamwenge district with a tropical rainforest vegetation and hills in the background. **B:** Study site in Central Uganda, Luweero district with tropical savannah type of vegetation

STUDY POPULATION AND DESIGN:

There were two study populations, humans and domestic animals (goats, sheep, cattle, pigs, and dogs). The populations in western Uganda were chosen purposively, mainly focusing on communities that live in and around Kasyoha-Kitomi forest reserve and are engaged in artisanal gold mining in Kitaka gold mines, but have also experienced MVD outbreaks. These were either miners, their family members or persons living within 30 km of the Kitaka gold mine cave. Individuals and domestic animals living in villages that experienced previous MVD outbreaks in 2007 and 2012 were recruited for this study. These were compared with human and animal populations from Luweero district in central Uganda.

All apparently healthy humans and livestock at the time of sampling were eligible for the study. Individuals that had a fever or showed symptoms of a VHF at the date of sampling were excluded and referred to the nearest health facility for treatment. The study design was a retrospective cohort, sampling individuals at present and tested them to see if they were exposed to filovirus in the past. Individuals from western Uganda acted as the "exposed group" for Marburg virus since they have experienced MVD outbreaks twice and live in an environment of confirmed bats with Marburg virus. Individuals from Luweero district were considered as the "exposed group" for ebolaviruses since they have experienced EVD

outbreaks twice and “unexposed group” for Marburg virus as no MVD has ever been reported in Luweero district.

SAMPLE SIZE, SAMPLING PROCEDURE, INCLUSION, AND EXCLUSION CRITERIA

For the exposed groups, a purposive sampling procedure was used with a snowball approach. Participants were questioned to learn who currently works, or used to work in Kitaka mine caves, and the recruited miners were questioned to learn of additional miners or ex-miners. All recruited miners and their family members, who were willing to participate, were included in the study. In Luweero district, random sampling of villages was employed. Once a village was selected, the investigators traveled to the location of the primary trading post at the village’s centre, and participants were chosen following the EPI method (Bennett et al., 1991). In this approach, the starting point at the village level was the centre of the village, then a bottle or a pen would be tossed to select the household to start with. The household in the direction of the head of the bottle or pen was the starting point. Then the next household was the nearest household to the previous household till the required number for that village was done. Purposive sampling was done mixed with convenient sampling because of the terrain and lack of a sampling frame. In each household, one person, usually the head of the household was studied, unless for purposively chosen risk groups such as miners and their family members. The animal herds were sampled according to their herd sizes, most of the herd sizes were below 15 animals with an average herd size of 4-5 animals. For large herd sizes more than 15 animals, only 25% of the herd was sampled.

Participants that consented to inclusion in this study, were asked to complete a questionnaire and give their answers verbally. One blood sample (minimum 4 ml) was collected in EDTA tubes from each participant and their animals for serological testing for filovirus IgG antibodies. Sample sizes were estimated by using Stata (Stata/ SE for Windows, StataCorp, College Station, TX). The total sample size for humans was determined to be 500; estimating a 14% prevalence of filovirus infection in the exposed group, 5% prevalence in the unexposed group, as well as a 95% confidence interval, 80% power, and a ratio of 2 controls to each exposed person. For domestic animals, the sample size was estimated as 865, assuming the seroprevalence of 30% in high-risk areas and 20% seroprevalence in low-risk areas. Blood samples were collected in EDTA vacutainer tubes following standard procedures by trained

biomedical personnel (Figure 7). The blood samples were aliquoted, kept under cold chain in nitrogen tanks and taken to the Uganda Virus Research Institute-Viral Haemorrhagic Fevers Laboratory at Entebbe and kept at -80 °C till testing.

Figure 7: Blood sample collection. **A**: Collecting a blood sample from a human. **B:** Blood sample collection from a goat.

QUANTITATIVE DATA COLLECTION.

Research assistants were trained to use a structured questionnaire to collect data. Participants were asked to give a written consent, after the objectives of the study were explained to them before the questionnaire could be administered. The questionnaire consisted of three sections, socio-demographic characteristics, practices that predispose people to EVD and MVD, and knowledge and attitude questions. Close-ended questions were used to assess peoples' knowledge and attitudes on transmission, risk factors, prevention and control, causation, signs and symptoms and treatment of MVD and EVD. To assess peoples' knowledge and attitude towards EVD/MVD, each participant's answers to these questions were scored. The knowledge and attitude score for each study participant were used to compute the percentage scores out of a total score of 34 and 20 respectively. The validity of the knowledge and attitude questions was confirmed by an adequate Cronbach's alpha internal consistency measured at 0.90. An animal data collection form was filled for each animal that was sampled.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data were entered into EpiInfo software, where a univariable analysis was done and later exported to Stata (Stata 13/ SE for Windows, StataCorp, College Station, TX) for further analysis. For knowledge and attitude study, a cut-off point was set based on percentage knowledge and attitude distribution, and median scores as was described in other studies (Iliyasu et al., 2015, Ali-Risasi et al., 2014). For knowledge scores, the median percentage score was 56%; with a bimodal curve distribution of the scores, hence those below a 56% score were categorised as having poor knowledge and those with 56% and above score as having good knowledge. Further, attitudes were classified as being negative if the percentage score was below the median score of 70% and positive if the median score was 70% and above.

Odds ratios were used to describe the relationships between outcome variables and independent variables in a logistic regression. Potential confounders were controlled for in multivariable logistic regression models constructed using a backward selection procedure using the likelihood ratio test (LRT) with a p-value=0.05 for keeping a variable in the model. Model evaluation was done using the Hosmer-Lemeshow test of goodness of fit and the area under the receiver operating curve (ROC).

PARTICIPATORY EPIDEMIOLOGY

Qualitative participatory appraisal techniques, also known as Participatory Epidemiology (PE) were used for qualitative data collection. Five (5) focus group discussions (FGDs) involving 50 participants were conducted (Figure 8). FGDs were held within rural communities that were affected by outbreaks, drawn mainly from survivors of EVD and their family members, community and opinion leaders, as well as other members of the community who were 18 years and above. The discussions involved both male and female respondents since gender disaggregation was not the focus of this study. To get an explicit knowledge of the community's knowledge and attitude towards EVD and MVD, three PE tools were used, simple ranking, proportional piling, and pairwise ranking. Discussions from FGDs were audiotaped with permission from informants and transcribed verbatim. Data generated through FGDs were analysed using conventional content analysis as reported by Hsieh and Shannon where qualitative data was merged into codes, categories and themes(Hsieh and Shannon, 2005).

Figure 8. Focused group discussions and proportional piling exercise with community members.

ETHICAL STATEMENT

Approval to conduct this study was obtained from Uganda Virus Research Institute's Research and Ethics Committee and Uganda National Council of Science and Technology (UNCST approval Nos: HS 1538 and HS 1940). Participants gave signed written consent to take part in this study. For participants under the age of 18 years, informed consent was provided by their parents or their guardians on their behalf.

LABORATORY METHODS

SEROLOGICAL TESTING OF HUMAN BLOOD SAMPLES

Human blood samples collected were tested by an Enzyme-Linked Immunosorbent Assay (ELISA) at Uganda Virus Research Institute (UVRI)/US Centers for Disease Control and Prevention (CDC) VHF laboratory in Entebbe, Uganda. The ELISA technique used on these blood samples was validated by US Centers for Disease Control and Prevention (CDC) on known positive and negative human blood samples with a sensitivity of more than 90% and specificity of more 90% (Ksiazek et al., 1999b).

Briefly, a gamma-irradiated lysate of Vero cells infected with either *Sudan ebolavirus*, *Bundibugyo ebolavirus*, *Zaire ebolavirus*, or Marburg virus was used as positive antigen, while the negative or control antigen had uninfected Vero cells. A volume of 100 µl of positive antigen diluted in PBS (Marburg Ag 1:3000 and Ebola Ag 1:2000 Dilutions) was applied on the upper half of the solid phase of a polyvinyl chloride microtiter plate and the lower half coated with 100 µl of negative/control antigen in PBS, and then incubated at 4˚C overnight. Antigen was removed from the well by washing three times with PBS-Tween. Samples were diluted 1:100 and 4-fold through 1:6400 in 5% skimmed milk in PBS-Tween and allowed to bind to the antigen. After washing, an anti-human IgG conjugated to horseradish peroxidase (HRPO) was applied and allowed to bind. The plates were washed, and the substrate ABTS (2.2'-Axinobis 3-ethylbenzothiazoline-6-sulfonic acid-diammonium salt) was added which in the presence of HRPO and hydrogen peroxide, is converted from a colourless liquid to an intense green colour with a maximum light absorption at 410 nm. The amount of colour developed is proportional to some IgG antibodies which has bound to the antigen on the solid phase. Optical density (OD) values at 410 nm were recorded on a microplate spectrophotometer. The OD value of the control antigen-coated well was subtracted from its corresponding viral antigen-coated well to yield adjusted OD value. A sample was considered positive when the adjusted OD value of either the 1:400, 1:1600 or 1:6400 dilution was higher than 0.2, and the sum OD value was higher than 0.95. A panel of 1 or 2 negative control sera and 2 or 3 positive control sera were run each time the assay was used.

SEROLOGICAL TESTING OF ANIMAL BLOOD SAMPLES

ELISA FOR DETECTION OF ANTIBODIES AGAINST EBOLA VIRUS

Animal blood samples were analysed at the Robert Koch Institute Berlin, Germany in Research Group 3- Epidemiology of Highly Pathogenic Microorganisms (P3). ELISA and a more specific Western Blot analysis were used to analyse the samples. A fourth assay, the Luminex based assay which is more sensitive, enabling us to detect multiple antibodies against several species of ebolaviruses (Ayouba et al., 2017) was done at Montpellier University, Montpellier, France. Figure 9 shows the schematic flow diagram showing the processes used to test the animal blood samples serologically.

Briefly, the screening ELISA using a recombinant glycoprotein (GP) as the detection antigen was the first, followed by two Western Blot (WB) analyses, firstly with GP only antigen followed by Virus-like particles (VLPs) containing GP, Nucleoprotein (NP) and matrix protein (VP40) and lastly a Luminex assay. ELISA techniques for diagnosing filoviruses have been described before. Some use authentic virus antigens made from virus-infected cells (Johnson et al., 1981, van der Groen et al., 1983, Ksiazek et al., 1999b, Ksiazek et al., 1999a) and others use the recombinant-based proteins diagnostic system for filoviruses (Saijo et al., 2001, Nakayama et al., 2010, Prehaud et al., 1998). Recombinant Ebola Virus Glycoprotein without the Transmembrane Region (EBOV rGpdTM) by IBT Bioservices, Inc Rockville USA) was used as the antigen in testing the domestic animal samples.

Briefly, 96 well ELISA plates (Optical readable micro test plates, sterile from Carl Roth GmbH + Co Karlsruhe, Germany) were coated overnight at 4°C with 100 µl of PBS containing 0.05 µg per well of the antigen after one hour of shaking at room temperature. The plates were washed three times with PBST using microplate washer (Tecan Trading AG, Switzerland). They were incubated with 200 µl of blocking buffer solution (5% non-fat dry milk powder in PBS) per well for 1 hour at room temperature under shaking at 180 rpm. The plate was knocked dry and 100 µl of the samples and negative control added in duplicates in 1:400 dilution as single point measurement. A positive control was added as serial dilution starting with 1:8000. Samples and controls were incubated on the plate for 2 hours under shaking at 180 rpm at room temperature. The plate was washed five times with PBST and incubated for one hour with secondary antibody (Horseradish Peroxidase Pure Donkey Anti-goat-from company). The plate was washed six times and incubated with TMB solution in darkness for 10 minutes. The reaction was stopped with 0.25M of Sulphuric acid. Plates were read in ELISA reader (Tecan Trading AG, Switzerland) at OD value 450 nm within five minutes after stopping the reaction. Each plate had four blank wells where no sample or controls were added. The OD values of the blanks were subtracted from OD value of wells with samples and controls. The Mean OD value of each duplicate was computed as well as the standard deviation (SD) and precision of measurement. The cut-off for positive samples was generated from mean OD value of negative control + 3SD, which was 0.2. Samples with mean OD value above 0.2 were considered positive. A subset of samples selected from within those with very low OD value, intermediate and high OD values from all three countries were taken into Western Blot (WB) testing.

WESTERN BLOT ANALYSIS

Western blot has been used in the detection of filovirus antibodies (Nakayama et al., 2010, Becker et al., 1992). For the study presented in this thesis, Western blotting was done in two steps; first samples were tested with GP antigen (EBOV rGpdTM) only, followed by virus-like particles (VLPs) expressing recombinant Ebola virus (EBOV) glycoprotein (GP), nucleoprotein (NP), and matrix protein (VP40). These VLPs are produced in sf9 insect cells through infection with recombinant Baculovirus from IBT Bioservices, Inc Rockville USA. Recombinant GP and VLPs were loaded on a 10% sodium dodecyl sulphate-polyacrylamide gel under denaturing and reducing conditions. The gel ran for 1 hour at 150V loaded with a coloured protein marker XXL DeLuxe (GeneOn GmH Ludwigshafen am Rhein, Germany). The antigens were later transferred from the gel to a polyvinylidene membrane at 15V for 20 minutes. Coated membranes were blocked with blocking buffer (5% non-fat milk powder in PBS) overnight at 4°C. Membranes were cut into small 3mm strips and placed on western blot plates. Samples were added in 1:100 dilution, negative control in 1:400 dilution, and positive control in 1:4000 dilution all in 1% non-fat milk powder in PBS, and incubated for one hour with shaking at room temperature. Additional controls for the VLPs rabbit anti-EBOV VLPs, anti-VP40 and anti-NP were used. Secondary antibodies (Horseradish peroxidase pure donkey anti-goat and anti-rabbit IgG-HRP) were incubated for one hour under shaking at room temperature and detection using TMB solution after incubation for 10 minutes in the dark. Only a subset of samples that had high OD values, intermediates, and low was included in Western blot analysis from Uganda, Ivory Coast, and DRC.

LUMINEX ASSAY

The Luminex assay used to test the goat blood samples has been described previously, when it was validated on human samples (Ayouba et al., 2017). Briefly, this is a serological assay based on Luminex technology for detection of antibodies against ebolaviruses. It is a sensitive and specific high-throughput serological assay that is important in epidemiological surveys. This multiple analyte profiling technologies is a flow cytometry-based system that allows fast and simultaneous detection of up to 100 analytes in a single well of a 96-well flat-bottom plate. Apart from detecting many antigens at a time, this technique is cost-effective regarding time and reagents and saves the scarce biological samples since it uses small volumes. In this study,

the Luminex assay was used to detect antibodies against four of the five species of *Ebolavirus* as has been explained by Ayoub et al., 2017. These include antibodies against *Zaire ebolavirus*, *Sudan ebolavirus, Bundibugyo ebolavirus* and *Reston ebolavirus*. A total of ten commercial Ebola virus recombinant antigens were used to assess antibodies in the goat samples. These include NP for Zaire, GP for Zaire Maying strain, GP for Zaire Kissidougou-Makona strain, VP40 for Zaire, NP for *Sudan ebolavirus*, GP for *Sudan ebolavirus*, VP40 for *Sudan ebolavirus*, GP for *Bundibugyo ebolavirus*, VP40 for *Bundibugyo ebolavirus* and GP for *Reston ebolavirus*.

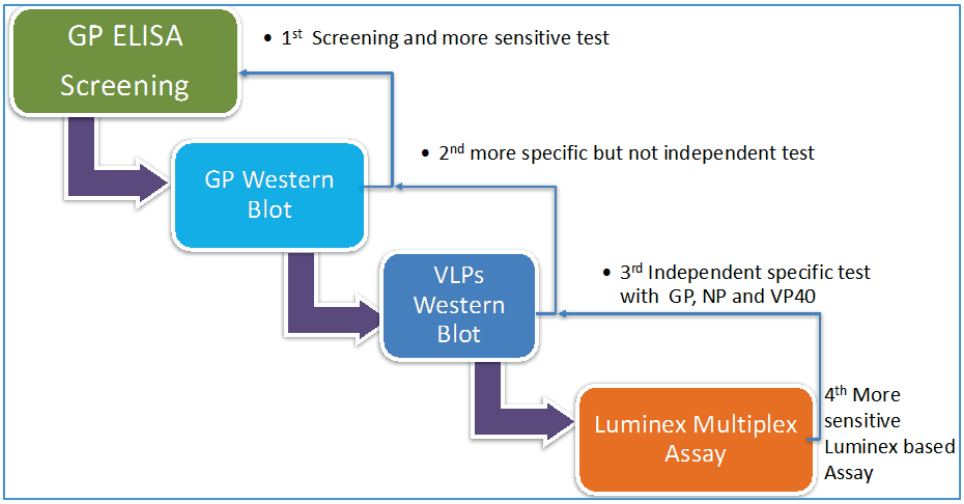


Figure 9: Goat blood sample testing algorithm**.**

MAIN RESULTS

SYSTEMATIC REVIEW AND META-ANALYSIS OF THE CASE FATALITY RATE AND SEROPREVALENCES (PAPER I)

After a full evaluation, 72 articles were included in the meta-analysis. 23 reported outbreaks of EVD, 12 reported outbreaks of MVD, 26 reported seroprevalence of Ebola virus and 11 reported seroprevalence of Marburg virus. Most of the seroprevalence studies reported both Marburg and Ebola viruses. The weighted average CFR of Ebola virus disease was estimated to be 65.0% [95% CI (54.0–76.0%), $I^2 = 97.98\%$] whereas that of Marburg virus disease was 53.8% (26.5–80.0%, $I^2 = 88.6\%$). The most severe species of *Ebolavirus* was *Zaire Ebolavirus* while *Bundibugyo ebolavirus* was the least severe. The overall seroprevalence of Ebola virus was 8.0% (5.0%–11.0%, $I^2 = 98.7\%$), whereas that for Marburg virus was 1.2% (0.5–2.0%, $I^2 = 94.8\%$). They were substantial heterogeneity in the published studies about CRF and seroprevalence of EVD and MVD. More details on these results, forest and funnel plots from the meta-analysis are in the attached Paper I.

RISK MAPS FOR FILOVIRUS OUTBREAKS IN UGANDA (PAPER II)

In Paper II, results of the ecological niche model show bats as potential reservoirs of filoviruses are distributed all over Uganda. Potential outbreak areas for Ebola and Marburg virus diseases were predicted in West, Southwest, and Central parts of Uganda, which corresponds to bat distribution and previous filovirus outbreak areas. Additionally, the ecological niche model predicted Eastern Uganda region and other parts that have not reported filovirus outbreaks before as potential filovirus outbreak hotspots. As expected for filovirus outbreaks, areas with steady rainfall and temperature seasonality were at high risk of having a filovirus outbreak. Rainfall variables were the most important in influencing model prediction compared to temperature variables. Risk maps and model evaluation details are in Paper II attached to this thesis.

KNOWLEDGE AND ATTITUDE TOWARDS EBOLA AND MARBURG VIRUS IN UGANDA (PAPER III)

Almost all of the participants (96.2%) had heard about EVD and MVD, 43.5% reported to know how to identify a suspect case of EVD and MVD, the most known clinical symptom for EVD and MVD was bleeding at 54.3%, and 28.2% reported to know a survivor of EVD and

MVD. On the mode of transmission, 51% knew how EVD and MVD are transmitted. A total of 62.8% (465/740) reported to know how EVD and MVD could be controlled and prevented with 52.8% (362/686) saying by avoiding sick people, 39.4% (270/686) by avoiding contact with animals and 11.1% (76/686) by vaccination. Only 4.5% (24/531) knew that a virus causes EVD and MVD, 58.7% thought that it is caused by wildlife such as non-human primates whereas only 1.1% attributed it to witchcraft. Regarding attitude, 87.3% (646/740) of participants believed that EVD and MVD exist, 52.7% (386/733) would not relate with a survivor of Ebola or Marburg virus disease.

Overall, out of 740 respondents, 48.5% (359/740) were categorised as being knowledgeable about Ebola and Marburg virus diseases, whereas 60.5% (448/740) had a positive attitude towards control and prevention of Ebola and Marburg virus diseases. The mean knowledge and attitude percentage scores were 54.3 (SD=23.5, 95% CI=52.6-56.0) and 69.9 (SD=16.9, 95% CI=68.9-71.1) respectively. Being male (AOR=1.9, 95%CI; 1.4-2.6), a miner (AOR=2.6, 1.7-3.8), attaining secondary (AOR=3.8; 2.3-6.3) and post-secondary (AOR=8.4; 2.5-27.5) levels of education were identified as predictors of knowledge about EVD/MVD from the logistic regression model. Qualitative data revealed that communities describe Ebola and Marburg virus diseases as very severe diseases with no cure and spread very fast. Respondents reported fear and stigma suffered by survivors, their families and the broader community due to this disease. People believed that Ebola and Marburg viral diseases kill instantly, cause chaos, and are more severe than HIV. There is much fear when the word "Ebola" is mentioned as it is considered a terrible disease.

"*When I hear Ebola, I lose strength because it kills instantly,*" said one of the participants in one of the FGDs. "*When you get Ebola, your life ends there,*" retorted another. More on participatory epidemiology techniques results, figures and tables can be found in Paper III attached to this thesis.

SEROPREVALENCE OF FILOVIRUSES IN HUMANS FROM SELECTED DISTRICTS IN UGANDA (PAPER IV)

Overall, 724 individuals were sampled, 433 (59.8%) from the exposed region in Western Uganda (Ibanda and Kamwenge districts) and 291 (40.2%) from the unexposed district of Luweero in Central Uganda. The median age was 33.0 (3-82), and 85.6% (620/724) of the

sampled people were ≥ 20 years, while 54.1% (391/724) were male. 71.6% of participants had primary school education or less, and 67.7% were farmers followed by miners at 22.2%. Other practices identified that could be risk factors for filovirus infection included going inside mines (19.1%), contacts with bats in the mines (34.7%), owning domestic animals (77.8%), hunting (3.9%), eating bush meat (47.9%), cleaning dead bodies at funerals (12.5%), going to the forest frequently (66.3%) and having bats in the house (56.2%).

In total, 2.6% (19/724) of the individuals tested had IgG antibodies reactive to filoviruses. Eighteen individuals had *Sudan ebolavirus* IgG antibody seropositivity (2.5%, 18/724), and one person had IgG seropositivity to both *Sudan ebolavirus* and *Bundibugyo ebolavirus* (0.1%, 1/724). One person had IgG antibody seropositivity to Marburg virus (0.1% 1/724). No individuals had IgG antibody against *Zaire ebolavirus*.

Study participants from Western Uganda who are exposed to mining activities and live around forest reserves were more likely to be seropositive (3.7%) for filoviruses compared to those from Central Uganda in Luwero district (1.1%) (OR=3.6, 95% CI; 1.1-12.2). However, this was not statistically significant after controlling for gender, age, education level, and occupation (OR=2.0, 95% CI 0.7-6.2). The risk factors for being seropositive for filoviruses were mining as an occupation (OR=3.4, 1.3-8.5), being male (OR=3.1, 1.01 - 9.5), being a family member of a miner (OR=3.3, 1.2-8.9), going inside mines (OR=3.1, 1.2 - 8.2), cleaning a dead body (OR=3.1, 1.04 - 9.1) and contact with EVD/MVD suspects (AOR=3.9, 1.04 - 14.5). Frequent travel outside a persons' home district was shown to be protective (AOR=0.3, 0.1-0.7). Details of these results, tables and figures are in Paper IV attached to this thesis.

SEROPREVALENCE OF EBOLAVIRUSES IN DOMESTIC ANIMAL SPECIES (PAPER V)

RESULTS FROM TESTING OF DOMESTIC ANIMAL BLOOD SAMPLES

Figure 10 and Table 3 show the summary of the results of the blood samples from all the five species of domestic animals tested on ELISA by Ebola virus recombinant GP antigen. There is a significant difference between species regarding reactivity (P> 0.001)-Fig 10A. Only cattle did not have detectable levels of OD values, meaning no or less reactivity against Ebola virus GP antigen. Pigs and goats had higher levels of reactivity against GP antigen with median OD

value at 0.69 (0.08-3.07) and 0.33 (0-3.35) respectively. Dogs and sheep also show reactivity against GP antigen with median OD value of 0.24 (0.02-3.35) and 0.33 (0.04-1.34) respectively (Figure 11B).

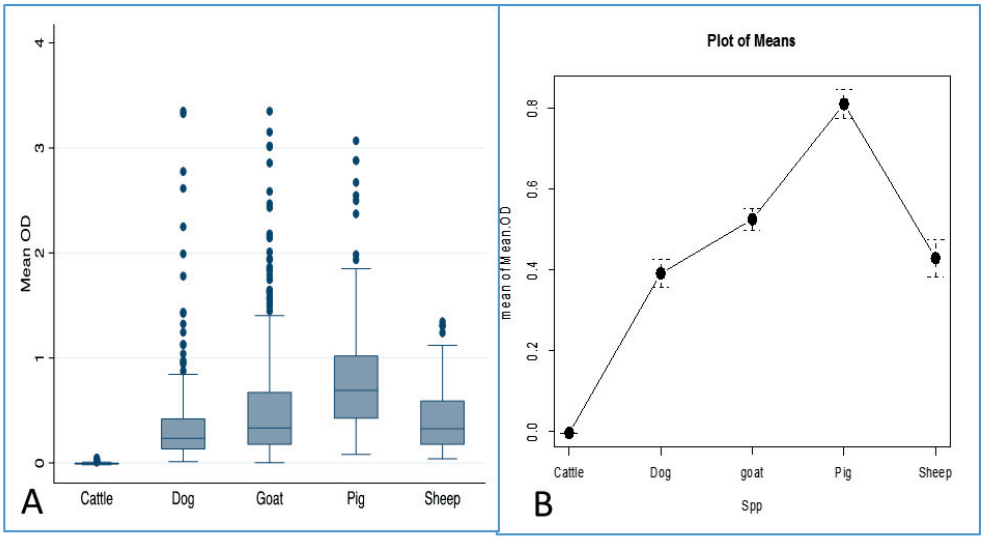


Figure 10: Mean OD values of domestic animal samples reactivity against GP antigen in ELISA. **A**: Box plot of mean OD values of all species tested. **B**: Plot of means of mean OD value of all the species.

Table 3: Summary statistics of Mean OD values of sampled species

Species	N	Average Mean OD value	S.D.	Minimum	Q25	Median	Q75	Maximum
Dog	208	0.39	0.5	0.02	0.14	0.24	0.42	3.35
Pig	227	0.81	0.53	0.08	0.43	0.69	1.02	3.07
Goat	399	0.52	0.55	0	0.18	0.33	0.67	3.35
Sheep	54	0.43	0.33	0.04	0.18	0.33	0.59	1.34
Cattle	336	0	0.01	-0.02	-0.01	-0.01	0	0.05

Due of lack of proper controls for ELISA, further testing was done to confirm these results. The paragraph below presents goat blood sample results that were tested further with other serological techniques other than ELISA.

RESULTS FROM TESTING OF GOAT BLOOD SAMPLES

From the ELISA test, 70% (282/399) of tested samples had mean OD value above 0.2 which was the cut off for negative samples. Results from three tests were combined (ELISA, Western blot with GP only, Western Blot with VLPs) to make an overall assessment of whether a sample was positive or not. Given these results, the cut-off for ELISA was shifted to 0.55 from 0.2, which gave a seroprevalence of 31.3 % (125/399).

For those samples that were run on WB, 50% (36/72) were considered positive. This was based on ELISA cut off OD value of 0.55 and above being positive on any of the two WBs. GP only WB, 37.5 % (27/72) were considered positive, GP in VLPs WB-69.4% (50/72), NP in VLPs WB-72.2% (52/72) and VP40 in VLPs-6.9% (5/72). It can be seen that NP and GP antigens embedded within the VLPs are more sensitive than VP40 and the GP only antigen in Western blot.

In the Luminex assay, there were several samples that were reactive against the four ebolaviruses tested in this assay. There was more reactivity against GP *Sudan ebolavirus* than other antigens. GP antigens from all the four species of Ebola virus assessed in the multiplex assay were more reactive than NP and VP40. Also, there is a lot of cross-reactivity between the four *Ebolavirus* species investigated in this study, as shown in the Luminex assay. Because of lack of positive control, it was hard to develop a proper cut-off for positive or negative control.

Goats from Uganda had the highest seropositivity at 39.8 % (94/236), while goats from Ivory Coast were at 21.3 % (23/108) and goats from DRC were at 14.6 % (8/55). Females and adult goats were more likely to be seropositive given by the statistically significant odds ratios. See paper V for details on goat sample testing results.

DISCUSSION

CASE FATALITY RATES AND SEROPREVALENCE OF FILOVIRUSES

From the meta-analysis and systematic review in Paper I, the overall pooled CFR of EVD of 65% was lower than the commonly reported CFR of 90% (Allam, 2014) . This still shows that, despite substantial heterogeneity between studies, more than half of the individuals who contract EVD are likely to die from it. The pooled CFR of EVD reported in this thesis is similar to that reported by Lefebvre et al. 2014, who reported a CFR of 65% in a study done using the WHO database on EVD outbreaks (Lefebvre et al., 2014). EVD outbreaks with CFR higher than the pooled CFR reported here either happened a long time ago or were single case outbreaks where one person contracted the disease and died. Recent outbreaks have lower CFR, showing either a change in severity of ebolaviruses or improvement in outbreak response. The high pooled CFR of EVD in the DRC (89%) compared to Uganda (43%) may partly be due to differences in health care system and response mechanisms to outbreaks, but also the severity of the species of *Ebolavirus* involved. For example, Uganda has developed a surveillance system for detecting viral haemorrhagic fevers and epidemic response is immediate within hours of confirmation of a positive case (Borchert et al., 2014). However, it is also important to note that Uganda has been affected by the less pathogenic species of *Ebolavirus* (*Sudan ebolavirus* and *Bundibugyo ebolavirus*) compared to the *Zaire ebolavirus* in DRC.

Another significant finding of this meta-analysis was the variation in the severity and CFR among the pathogenic species of ebolaviruses. *Zaire ebolavirus* (pooled CFR=75%) was the most severe, followed by *Sudan ebolavirus* with a pooled CFR=53%, while *Bundibugyo ebolavirus* (pooled CFR=34%) was the least severe of the species. This finding is supported by McCormick et al., 1983 who showed that *Zaire ebolavirus* was more lethal to suckling mice than *Sudan ebolavirus,* which did not kill any experimental animal (Mccormick et al., 1983). The reasons for the severity of *Zaire ebolavirus* are unclear. Thus there is a need for further research to determine whether genetic differences are responsible for the variation in the pathogenesis of these species. However, the meta-regression did not show any influence on CFR of EVD by country of the outbreak ($p = 0.25$). This is probably due to the low power, given the few number of outbreaks that have been reported globally.

With the *Metaprop* command for meta-analysis of marginal proportions, it was possible to estimate the 95% confidence intervals for MVD pooled CFR as 61% (32–88%), slightly lower than the pooled CFR of EVD. The CI was very wide because of the few outbreaks and the low number of cases involved in MVD outbreaks as compared to EVD outbreaks. In this study, it was estimated that 8% and 1.2% of the apparently healthy people in areas of Central African countries are likely to be seropositive for Ebola and Marburg viruses respectively. This finding suggests that some people do get infected with filoviruses and are either asymptomatic or get a severe disease and make a full recovery without being detected by healthcare systems. The limitation of these effect estimates was the heterogeneity that was observed between studies. Studies with different study designs and from different countries are more likely to exhibit heterogeneity. Funnel plots and Beggs tests suggested that publication bias might have been present, meaning that studies with negative results about Ebola and Marburg viruses are less likely to be published, hence affecting the estimate of seroprevalence and CFR for EVD and MVD.

SPATIAL MODELING AND RISK MAPPING FOR FILOVIRUS OUTBREAKS

In Paper II, seven environmental variables were used to develop a risk map for filovirus outbreaks in Uganda. Variable contribution assessment to the risk map prediction model showed that rainfall variables were the most significant predictors. The importance of rainfall or precipitation and moderate to high temperature was highlighted by Peterson et al.. (2004) when they modelled filovirus distribution in Africa using GARP model (Peterson et al., 2004, Peterson et al., 2006). Rainfall is essential, for the apparent reason that it provides water which is crucial for bat survival (Russo et al., 2012, Adams and Hayes, 2008). Rainfall also supports the development of fruiting trees that provide roosting areas for bats as well as food for fruit bats. Uganda is endowed with many water bodies and several rainforests, and hence bat distribution tends to be all over the country. Since bats are hypothesised to be reservoirs for filoviruses; their distribution tends to correlate with that of filovirus predicted niches (Paper II).

Temperature and rainfall seasonality were the most critical environmental variables contributing to the spatial prediction model for the Ebola and Marburg viruses. Seasonality has been found to be essential in outbreaks of filoviruses, especially MVD as was reported in an ecological study by Amman et al. (2012). In the Amman et al. (2012) study, outbreaks of MVD

are associated with the birthing seasons of bats, when the virus circulation was high. This is further validated by a high contribution (68.2%) of temperature seasonality variable into the MVD outbreak prediction model. The relative probability of the presence MVD outbreak is higher (80%) at very low-temperature seasonal variation. Therefore, areas with less variability in monthly temperature and rainfall are more likely to experience MVD and EVD outbreaks. The areas shown on the risk maps with a high relative probability of the presence of an outbreak are mainly in the South, the West and Central Uganda that have minimum temperature and rainfall variations compared to North Eastern Uganda that is not predicted for filovirus outbreaks despite the presence of bats.

The predictions show that a significant part of Uganda, a country of 34 million people, is at risk of a filovirus outbreak. This is more so in the Lake Victoria basin districts and in the Albertine Rift valley region districts and the areas that occur in between. The Albertine Rift valley region provides a variety of habitats characteristic of the East African savannahs and the West African rainforests that are suitable for reservoirs of filoviruses. According to Uganda National Meteorological department, these are the areas that receive near or above average seasonal rainfall, and seasonal temperature variations are minimal (UNMA, 2016). Indeed, six filovirus outbreaks have been reported in this region, one caused by *Bundibugyo ebolavirus* in Bundibugyo district in the plains of Rwenzori mountains (Wamala et al., 2010), *Sudan Ebolavirus* in Kibale district (Albarino et al., 2013) and four outbreaks of Marburg virus, all linked to the Python cave and Kitaka mine caves in Kamwenge, Ibanda, and Rubirizi districts (Knust et al., 2015, Adjemian et al., 2011, Centers for Disease and Prevention, 2009, Timen et al., 2009). This remains a high-risk area with cross-border movement between Uganda and DRC where another EVD outbreak happened in 2012 in the neighbouring Isiro region (Kratz et al., 2015). The Albertine Rift valley of East Africa needs to remain under heightened surveillance, because oil exploration will be taking place bringing an invasion of virgin lands by humans and interaction of wildlife and humans. All outbreaks of Marburg virus disease in Uganda that have been investigated, originate from the old gold mines found in Ibanda and Kamwenge districts (Adjemian et al., 2011, Knust et al., 2015) in the Western Rift Valley which validates the MVD distribution model, as it shows these as high-risk areas for filovirus outbreaks. A similar finding was obtained in a recent model using MaxEnt as they predicted *Sudan ebolavirus* to occur in North Western Uganda between Lake Albert and Lake Victoria (Peterson and Samy, 2016).

We also see areas that have not had EVD outbreaks before, such as West Nile region, predicted potential areas for EVD outbreak. These include areas along the River Nile and areas bordering South Sudan and DRC. These areas receive average annual rainfall between 100-120 mm and are endowed with high vegetation cover and water bodies, all of which make the region conducive for reservoirs of filoviruses. Other areas that have not had outbreaks of filoviruses include areas such as the Eastern region of Mbale, Busia and Tororo districts near the Mt. Elgon regions bordering with Kenya. This could be due to the presence of suitable conditions for survival of putative reservoirs of Ebola and Marburg viruses. An outbreak happened in neighbouring Kenya in Kitum Cave (Johnson et al., 1983b, Smith et al., 1982). These newly detected hotspots need to be kept under surveillance for early outbreak detection and response.

This study builds on several filovirus risk mapping efforts (Peterson et al., 2004, Peterson et al., 2006, Peterson and Samy, 2016, Pigott et al., 2014, Pigott et al., 2015, Pigott et al., 2016), all of which have been done at the continental level of Africa. Their work was more ecologically oriented and focused on identifying the ecological niche of species. They lacked country-specific details that have been included in this thesis with a bias in public health surveillance and outbreak detection rather than ecological niche identification. For public health surveillance of a country like Uganda, all filovirus species (Marburg virus, 5 *Ebolavirus* species) are of public health importance. This makes the models presented here more sensitive, as opposed to specific risk map and hence a more useful tool to the surveillance activities. There is already enough evidence of filovirus outbreaks in Uganda, especially areas predicted by these models. Focused surveillance needs to be done in these areas and bring additional surveillance in other new predicted areas where there have not been outbreaks.

KNOWLEDGE AND ATTITUDES TOWARDS EBOLA AND MARBURG VIRUS DISEASES

Paper III demonstrates that EVD/MVD affected communities in Uganda were knowledgeable about EVD/MVD at 48.5% and 60% have a positive attitude towards control and prevention of these diseases. This is slightly higher than what has been found in similar studies about knowledge towards Ebola virus disease especially in West Africa (Snell et al., 2015, Schumacher et al., 2015, Patino-Barbosa et al., 2015, Patel et al., 2015, Olowookere et al., 2015, Highsmith et al., 2015, Gupta et al., 2015, Gidado et al., 2015, Alfaki et al., 2015, Iliyasu et al., 2015, Kobayashi et al., 2015). This may be because Uganda has had many more outbreaks of Ebola and Marburg viral diseases, which has led to continuous sensitisation of

communities to these diseases, hence change in attitude, behaviour and more knowledge gained. However, the proportion categorised as knowledgeable about EVD/MVD is still below average (48%), and more sensitisation is needed if future outbreaks are to be controlled in the shortest time possible. Community support and involvement represent a key in control and prevention of VHFs. Given that this survey was done in communities that had exposure to VHF outbreaks, the knowledge levels could even be lower in other naïve communities. There is still a significant proportion (51.5%) that is less knowledgeable and have negative attitudes (40%) towards control and prevention. One would have expected higher levels of knowledge given that the studied communities have experienced filovirus outbreak twice. Although the communities demonstrate much fear towards Ebola and Marburg viruses, this can be advantageous for control and prevention measures as communities will be motivated to act if in case an outbreak occurs. However, this fear becomes counterproductive as far as survivors are concerned. Disease stigma is still an issue as 53% of the respondents said they would not associate with a survivor for fear of contracting the disease. This was also observed in the 2001 EVD outbreak in Northern Uganda, as communities initially had their reservations about Ebola virus disease and survivors. However, after they had been explained to entirely by the health care workers, survivors were accepted and are living peacefully in their communities (Hewlett and Amola, 2003). It is still hard for communities to fully accept that people completely recover from infection with ebolaviruses and that they can easily mix and interact with the rest of the community as evidenced in this research and from the West Africa EVD outbreak experience (Lee-Kwan et al., 2014, Gidado et al., 2015).

Most participants mentioned that filoviruses spread fast, meaning they are highly contagious as was discussed in focused group discussions. Several modes of transmission were reported by the participants, which include contact with infected patients and contact or eating non-human primates and bats. This knowledge by the community is helpful during outbreaks in instituting control and prevention measures by health authorities. In communities that do not know modes of transmission, it would be hard to stop the spread of the epidemic as was seen in West Africa (Osungbade and Oni, 2014). However, we still have a few people who think that EVD and MVD are airborne, caused by witchcraft or by the malice of medical workers from foreign countries. This was also revealed in an anthropological study in one of the study areas in Luweero district (de Vries et al., 2016). Such misconceptions need to be addressed, because if taken on by opinion and community leaders as it happened in 2012 Luweero EVD

outbreak, they could hamper prevention and control measures. Sensitization of communities about filoviruses was the most effective means of control and prevention, suggested by participants. Unlike the survey that was done in West Africa, where watching television was the most common sources of information (Gidado et al., 2015), participants in this study preferred the use of community radios as the most efficient way of passing on information to the communities. This model involves the use of loudspeakers placed in community trading centres where announcements can be made.

Another big complaint that is social-cultural in nature was a failure by the community to bury their loved ones. This needs to be addressed during outbreaks so that communities can feel that their loved ones have been buried properly. In some African cultures people believe that if someone is not buried properly, he/she will come back in real life to haunt the living family.

Being educated beyond primary level was the most critical determinant of knowledge and attitude towards EVD/MVD. Education is a crucial determinant of knowledge, especially concerning health and health-seeking behaviours and it has been found to influence people's knowledge about EVD in Nigeria (Iliyasu et al., 2015).

Gender disparities were explored in this study using a proportional piling technique. Although many studies show that VHFs tend to affect more females than males because of their gender roles (Diggins and Mills, 2015) this study revealed that almost all men and women are affected equally. However, from the FGDs, men were indicated as more likely to be index cases than women because of their risky behaviour and gender roles such as hunting, clearing land for agriculture and going into the forest for several activities. As the outbreak progresses, women tend to be more likely to be affected since they are more into caring for the sick hence having higher chances of being infected.

Results from this study may not be generalised to the communities in the whole of Uganda. Communities were selected purposively because of their previous experience with Ebola and Marburg outbreaks. Communities that have experienced outbreaks are more likely to have received education through social mobilisations that happened during outbreaks, and hence appear to be more knowledgeable than other communities that have not experienced outbreaks.

SEROEPIDEMIOLOGY OF FILOVIRUSES IN HUMANS

For the first time a seroprevalence of filoviruses in Uganda in apparently healthy individuals is reported (Paper IV). These findings point to the hypothesis that filovirus infections may occur in Sub- Sahara African countries and go undetected by the healthcare systems. The study also shows that people who live near caves inhabited by fruit bats are at higher risk of infection with filovirus compared to other populations. This could lead to more extensive epidemics as was seen in West Africa in 2014.

This study reports 19 individuals that were IgG antibody seropositive representing 2.6 %. Out of these 19, 18 were IgG antibody seropositive for *Sudan ebolavirus,* and one was seropositive for Marburg virus. Of the 18 seropositive for *Sudan ebolavirus*, one was also seropositive for *Bundibugyo ebolavirus.* This is slightly lower at 3% and 8% for pooled prevalence reported in meta-analyses of seroprevalence of Ebola virus performed in other parts of the world . Also, following the West Africa outbreak, reports of asymptomatic infection in West African populations have been suggested (Glynn et al., 2017, Richardson et al., 2016). This study also reported a lower seroprevalence of Ebola virus than reported in other studies in neighbouring Democratic Republic of Congo (DRC) (Busico et al., 1999, Becquart et al., 2010a, Heymann et al., 1980, Commission, 1978, Nkoghe et al., 2011, Van der Groen and Pattyn, 1979), Central African Republic (Saluzzo et al., 1981, Meunier et al., 1987, Johnson et al., 1993c, Johnson et al., 1993d, Nakounne et al., 2000, Gonzalez et al., 2000), Gabon (Georges et al., 1999, Bertherat et al., 1999, Lahm et al., 2007, Heffernan et al., 2005), Sudan (Baron et al., 1983), Madagascar (Mathiot et al., 1989), Liberia (Meunier et al., 1986) and Cameroon (Bouree and Bergmann, 1983, Paix et al., 1988), but showed higher seroprevalence than that reported in Nigeria (Tomori et al., 1988), Germany (Becker et al., 1992) and Kenya (Johnson et al., 1983a). Only one Marburg virus seropositive person was confirmed in this study, and this was much lower than has been reported in other Marburg virus seroprevalence studies (Van der Waals et al., 1986, Gonzalez et al., 1989, Johnson et al., 1983a, Mathiot et al., 1989, Becker et al., 1992, Johnson et al., 1993a, Johnson et al., 1993b).

The study also reports for the first time the sero-reaction of *Bundibugyo ebolavirus* in one individual, while no individuals showed sero-response to *Zaire ebolavirus*. These variations in seroprevalence could be due to differences in filovirus ELISA testing protocols. The test, developed by CDC that was used in this study has been shown to be more specific than other

filovirus serological tests used in previous filovirus seroprevalence studies (Ksiazek et al., 1999b).

There is a higher seroprevalence from Westen Uganda study groups (exposed) at 3.7% (16/433) compared to Central Uganda study group (unexposed) at 1.1% (3/291). Although this difference was confounded by the fact that in the exposed study participants, most are miners who had an even higher prevalence of 5.6% compared to non-miners (1.8%), (OR=3.4, 1.3-8.5). A higher seroprevalence of Marburg virus was expected, but the opposite was observed with *Sudan ebolavirus* seroprevalence being higher than that of Marburg virus. There may be a reservoir for *Sudan ebolavirus* in Kitaka mines and inhabiting the areas around the Kasyoho-Kitomi reserve ecosystems that has exposed these individuals, especially gold miners to filoviruses such as *Sudan ebolavirus*. These findings are consistent with what was reported in another filovirus serological study by Nkoghe *et al.,* (2011) in rural Cameroon and Gabon where the prevalence of Ebola virus was higher in populations near forests (Nkoghe et al., 2011, Becquart et al., 2010a, Bouree and Bergmann, 1983). Another study in Gabon found that pygmies, who are forest dwellers, had a higher ebolavirus seroprevalence than other populations at 7.0% compared to non-pygmies (4.2%) (Gonzalez et al., 2000). This still indicates that communities that live in the forested areas, like the ones studied here in Uganda are at higher risk of infection with filoviruses compared to those living in more developed or non-forested areas. Being male was associated with a high risk of being seropositive compared to being female, partly because men are more likely to be miners and go inside the mines and the forests hence acquire infection and become index cases which bring the infection to the rest of the family members. Participating in a funeral, especially cleaning or preparing the dead body, was highly associated with being seropositive for a filovirus. This has been widely reported in outbreaks of filoviruses as burials and funeral rites amplify these outbreaks.

SEROEPIDEMIOLOGY OF EBOLA VIRUS IN ANIMALS

Paper IV describes for the first time, the testing and detection of IgG antibodies against ebolaviruses in domestic animals (goats, sheep, cattle, pigs). Although we also tested dogs, this has been done before in Gabon (Allela et al., 2005). We see that apart from cattle, there was a reactivity against Ebola virus GP recombinant antigens in goats, pigs, sheep and dogs. This could indicate that livestock is exposed to filoviruses and could be a potential source of

infection to humans. More research is needed to investigate this reactivity to rule out cross-reactivity.

Goat samples were tested further. A domestic goat is a close species to a duiker which belongs to the same family as goats-*Bovidae.* A duiker was found to be positive for Ebola virus by RT-PCR (EBOV) in a wildlife monitoring study in Gabon and Republic of Congo (Rouquet et al., 2005). The presence of IgG antibodies against ebolaviruses in goats could be a sign that these species could be infected with the filoviruses, and they could be potential reservoirs or at least are potential sources of infection to man. There were minor differences in seroprevalences between countries Uganda, DRC and Ivory Coast, perhaps due to numbers sampled in these countries, but also the differences in interaction between probable wildlife reservoirs of ebolaviruses and domestic animals. In Uganda for example, there is high human population density, and humans tend to invade the wildlife more than in DRC and Ivory Coast. They do so with their animals, hence increasing exposure of these domestic animals to filoviruses. Goats, in particular, are browsers and are more likely to feed higher in shrubs, tree leaves and fruits which could have been exposed to bats saliva, as research has shown that filoviruses are more likely to be shed orally in saliva than other routes of viral shedding (Amman et al., 2015b). There is a significant difference in seropositivity between male and female goats but not between breed. Also, lactating and pregnant animals had significantly higher antibody responses and seroprevalences compared to other reproductive status of the animals. This is consistent with what has been found in bats where the infection was high around birthing seasons (Amman et al., 2012). It seems there is a relationship that needs to be investigated concerning giving birth and infection with filoviruses. Another risk factor for seropositivity was age, where adult goats were four times as likely to be seropositive as juveniles, maybe because, with older goats, there are higher chances of being infected and hence seroconversion. IgG antibodies can last for as long as 14 years in humans as has been shown for *Sudan ebolavirus* survivors in Uganda (Natesan et al., 2016*)*. This high seroprevalence in goats could be due to accumulated cases over time, but this should be interpreted with the lifespan of domestic goat in question as these goats are not kept for long, and they are usually slaughtered in case of visitors or sold for income.

The limitation of this study, however, is the challenge of cross-reactivity of IgG antibodies within filoviruses species. In studies done on filovirus survivors and monkeys vaccinated with VLPs (Kamata et al., 2014, Natesan et al., 2016), antibodies against *Sudan ebolavirus* would

cross-react with those of *Bundibugyo ebolavirus,* especially NP. Also in these studies, there was a cross-reactivity of Marburg virus antibodies with those of ebolaviruses. In this study as well, there was a lot of cross-reactivity between different ebolavirus species. Although we detected signals generated against recombinant Ebola virus proteins GP, NP and VP40, we cannot be sure that these signals are generated against only ebolaviruses. It strongly recommends other diagnostic techniques such as neutralisation tests or PCR to confirm which species of filoviruses species antigens are involved. Here, recombinant Ebola virus proteins were used to detect antibodies against Ebola virus in goats in three African countries using ELISA, Western Blot and Luminex Assay Technology. These proteins has been evaluated and found to be useful in seroepidemiological studies (Prehaud et al., 1998, Saijo et al., 2006).

METHODOLOGICAL LIMITATIONS

Research in filoviruses has been on a low-key since the 20th century, gaining some attention in the 21st century, especially after the EVD outbreak in West Africa in 2014. This is unlike HIV and Malaria which has attracted funding and have been well studied. The reason for this has been mainly few outbreaks of filoviruses and the ability of the diseases to wane out without the potential for a pandemic and occurring in very remote areas especially in East and Central Africa. The primary challenge we faced in conducting research presented in this thesis was a lack of validated methods for filovirus research, especially diagnostic and laboratory methods for testing animal samples. No single method, for example, has been validated to test for antibodies against filoviruses in domestic animals such as goats, cattle, and sheep. Serological studies are significant for epidemiology and surveillance, and they help in assessing risk factors that can feed into control and prevention. However, as a result of the EVD outbreak in West Africa, new methods are being developed to test for antibodies in animals, especially pigs, but most are focused on human testing. Even those focused on animal studies, there is a significant challenge of not having positive and negative controls which are very important for validation of experiments and comparisons. For example, it was hard for us to get controls for testing goat samples unless those that have been experimentally infected with recombinant filovirus antigens which may not have the same biological environment with the test samples. Using controls from Europe, for example, may not be proper for validating test samples from Africa because of different exposures to different molecules in the environment.

Another methodological challenge is the reported cross-reactivity within filovirus species and cross-reactivity with other pathogens. There is a lot of scepticism within the scientific community about filovirus serological tests. Lack of validated multiplex assays for testing filoviruses in animals is a big challenge. *Ebolavirus* alone has five species and Marburg virus has two species which have potential to cross-react with each other. Assays that test one analyte at a time are expensive regarding time and need for resources. Luminex multiplex assay that had been validated on human blood samples was used on goat samples with exciting results (Ayouba et al., 2017). There are efforts in this direction, but as mentioned earlier, this is still focusing on human research and more need to be developed for testing animals if we are to have a chance of identifying a reservoir for ebolaviruses.

Filovirus diseases are rare diseases, and this presents a challenge when designing epidemiological studies. Sampling for a rare disease is not as clear because of the need for a large sample size to increase chances of detecting a disease. This is more so in the search for a reservoir for filoviruses. How many species do you need to sample and in what numbers (sample size) to be able to detect the infection? Although there are methods to determine sample sizes of this nature, usually the outcome is subject to time and resources to test all the samples. A good example was during the research for this thesis, five species were sampled (goat, sheep, pig, dog and cattle), but only goat samples could be tested to completion because of lack of resources and the lack of validated tests for these animals. Filoviruses are also considered biosafety level four pathogens; hence not many laboratories are willing to test for them or develop diagnostics because of fear of infection due to lack of proper biosafety and biosecurity facilities. The situation is worse if the laboratory is located in developing countries because of the bioterrorist potential of filoviruses. All filovirus laboratories located developing countries are managed by foreign countries that have biosecurity interests focused on biocontainment rather than research development. So, this makes research even harder in countries where these outbreaks happen because samples have to be transported abroad for culture or sequencing which is even more expensive. Developing capacity in developing countries in Sub-Saharan Africa where these outbreaks happen will greatly help in controlling these outbreaks.

For the other studies presented in this thesis, we found no major methodological limitation, and we believe that the meta-analysis, our climate models and knowledge and attitude studies are representative of the real situation in Uganda at the time our studies were undertaken.

CONCLUSIONS, RECOMMENDATIONS, AND FUTURE RESEARCH

CONCLUSIONS AND RECOMMENDATIONS

The CFR for Ebola and Marburg viruses is still moderately high but not as high as has been reported in the media and other publications. The CFR of EVD and MVD is higher in countries with inadequate disease surveillance systems and health care service delivery in general. This calls for an improved surveillance system that will enhance early detection and response to these filovirus outbreaks to avoid pandemics. The presence of seropositive individuals in apparently healthy populations indicate that cases go undetected by the health care system in affected countries; further calling for robust surveillance for Ebola and Marburg viruses.

Using Ecological niche modelling, it can be seen that many regions in Uganda are hot spots for filovirus disease outbreaks and hence should be a focus in filovirus surveillance for early detection. In the absence of a known reservoir, risk maps can help focused surveillance and early detection to avoid a global catastrophe as it happened in West Africa in 2014. It is recommended that this risk map is used in targeted sampling for filovirus reservoir.

The knowledge and attitude study revealed that communities in Uganda that had been affected by filovirus outbreaks are slightly knowledgeable and have a good attitude towards control and prevention of EVD. Formal education is a significant predictor of knowledge and attitude towards filoviruses. Communities could identify the suspect cases and are aware of the modes of transmission, and they suggest sensitisation as the best approach for control of filovirus outbreaks. Although Uganda's health sector has developed preparedness plans to respond to filovirus outbreaks, the level of knowledge about filoviruses is still low and needs to be improved. The public health sector could enhance communities' knowledge and attitude by supplying more educational materials and conducting health education for epidemic preparedness and using proper communication channels as proposed by the communities.

Seroepidemiological studies in both humans and domestic animals showed that there are asymptomatic infections that go undetected by the health care system in Uganda. Increased surveillance is critical in averting future filovirus pandemics. This thesis reports for the first time the presence of antibodies against several recombinant antigens in goats, pigs, dogs, and

sheep in Uganda. This needs to be investigated further to ascertain if goats and other livestock are potential reservoirs of filoviruses by doing more research.

FUTURE RESEARCH

Although some research has been done on Ebola virus dynamics (Agusto, 2017, Ngwa and Teboh-Ewungkem, 2016, Wiratsudakul et al., 2016, Guo et al., 2016, Berge et al., 2016, Althaus, 2015, Xia et al., 2015, Althaus et al., 2015, Legrand et al., 2007, Chowell et al., 2004), there is need for more research in this areas. From the meta-analysis of CFR presented in this thesis (Paper I), it is evident that the CFR of the three most pathogenic species of Ebolavirus is different (Nyakarahuka et al., 2016), but we do not know if the Basic Reproduction Number (R_0) of these species differ. This is important in estimating the intensity of outbreaks and response preparedness. R_0 of different species of filoviruses would also help in estimating the herd immunity since there seems to progress in Ebola virus vaccine production. We need to know the percentage of a given population that could be vaccinated to avert future outbreaks and Ebola and Marburg virus dynamics would greatly help in this area. The R_0 that has so far been estimated is of Ebola virus and *Sudan ebolavirus*, but not Bundibugyo *ebolavirus* and other filovirus species. Even the R_o estimates need to be updated given the changing disease dynamics. The rate of contact between individuals could differ from one culture to another hence affecting the transmission coefficient ß.

We developed a risk map for filoviruses in Uganda, but the ecology of filoviruses still need to be investigated, especially inclusion of other environmental factors such as vegetation, elevation and find out how social-cultural factors affect the distribution of filoviruses. Increased research in filovirus disease ecology will also help to identify the reservoir for these filoviruses as this is critical in instituting control and prevention measures. This is also related to research needed in assessing the impact of filovirus outbreaks especially for survivors of EVD and MVD. There is a need for longitudinal studies that look at the sequelae especially now the West African cohort of EVD survivors. The study of socio-cultural fears and stigma will allow design health promotion models that are filovirus-specific and other emerging pathogens.

As presented in this thesis, goats, sheep, pigs, and dogs could be playing a role in the epidemiology of filoviruses; there is need to study further these domestic animals and include other domestic species to find out if they are exposed to the filoviruses and if so, determine the

implications of this. If indeed these animals are exposed to filoviruses, and they are so much in contact with humans in Africa, why are the outbreaks of filoviruses not so many?

Molecular epidemiology of filoviruses is still demanding, and there was a debate of whether the virus that caused the outbreak in West Africa was indeed of *Zaire ebolavirus* species, but it was found out that it is a strain of *Zaire Ebolavirus,* a bit different from that in DRC and Gabon (Basler, 2017, Baize et al., 2014). We need more research to do a molecular characterisation of filoviruses circulating in Africa. We need more knowledge on which species or strains of filoviruses infect which hosts and what are their putative reservoirs. Can vaccination against one species of the filoviruses give protection against other species, because of reported cross-reactivity between species, although this presents a diagnostic challenge, it presents a disease control opportunity? Further study of unreported outbreaks or asymptomatic filovirus infection needs to be conducted. Do seropositive people against filoviruses present with a severe disease with clinical signs that were not detected by the health system or were they asymptomatic throughout the infection.

As already highlighted in the methodological challenges above, there is a need for research in developing diagnostic kits and laboratory methods that are user-friendly especially in underdeveloped countries for easy research and diagnosis of filoviruses for early detection. In conclusion, there is still a lot of unanswered questions on filoviruses some of which we tried to discuss in this thesis and more are beyond the scope of this thesis, as this thesis was more focused on epidemiology and public health point of view.

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APPENDIX I: ENCLOSED PAPERS

PAPER I

Nyakarahuka et al. BMC Infectious Diseases (2016) 16:708
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BMC Infectious Diseases

RESEARCH ARTICLE Open Access

How severe and prevalent are Ebola and Marburg viruses? A systematic review and meta-analysis of the case fatality rates and seroprevalence

Luke Nyakarahuka[1,2,5*], Clovice Kankya[2], Randi Krontveit[3], Benjamin Mayer[4], Frank N. Mwiine[2], Julius Lutwama[5] and Eystein Skjerve[1]

Abstract

Background: Ebola and Marburg virus diseases are said to occur at a low prevalence, but are very severe diseases with high lethalities. The fatality rates reported in different outbreaks ranged from 24–100%. In addition, sero-surveys conducted have shown different seropositivity for both Ebola and Marburg viruses. We aimed to use a meta-analysis approach to estimate the case fatality and seroprevalence rates of these filoviruses, providing vital information for epidemic response and preparedness in countries affected by these diseases.

Methods: Published literature was retrieved through a search of databases. Articles were included if they reported number of deaths, cases, and seropositivity. We further cross-referenced with ministries of health, WHO and CDC databases. The effect size was proportion represented by case fatality rate (CFR) and seroprevalence. Analysis was done using the *metaprop* command in STATA.

Results: The weighted average CFR of Ebola virus disease was estimated to be 65.0% [95% CI (54.0–76.0%), $I^2 = 97.98\%$] whereas that of Marburg virus disease was 53.8% (26.5–80.0%, $I^2 = 88.6\%$). The overall seroprevalence of Ebola virus was 8.0% (5.0%–11.0%, $I^2 = 98.7\%$), whereas that for Marburg virus was 1.2% (0.5–2.0%, $I^2 = 94.8\%$). The most severe species of ebolavirus was *Zaire ebolavirus* while *Bundibugyo Ebolavirus* was the least severe.

Conclusions: The pooled CFR and seroprevalence for Ebola and Marburg viruses were found to be lower than usually reported, with species differences despite high heterogeneity between studies. Countries with an improved health surveillance and epidemic response have lower CFR, thereby indicating need for improving early detection and epidemic response in filovirus outbreaks.

Keywords: Ebola virus disease, Marburg virus disease, Case fatality rate, Meta-analysis, Systematic review, Seroprevalence

Background

Ebola virus disease (EVD) and Marburg virus disease (MVD) are caused by filoviruses in the family *Filoviridae* and are both associated with high case fatality rates (CFR). The World Health organization (WHO) reports that the CFR of EVD ranges from 25.0 to 90.0% while that of MVD ranges from 24.0 to 88.0% [1]. In the early phases of a major Ebola outbreak in West Africa, CFR was reported to be 70.8% [2]. The CFR of EVD seems to be species dependent with *Ebola Zaire* and *Ebola Sudan* species being most pathogenic (with a reported CFR of 100%), while *Ebola Bundibugyo* appears to have a lower CFR at 34% [3]. A recent study by Lefebvre *et al.* that used data from WHO database estimated the CFR of EVD to be 65.4% irrespective of the Ebola virus species [4]. A few studies have tried to pool the CFR of EVD and MVD, but did not use the meta-analysis approach [5].

* Correspondence: nyakarahuka@gmail.com; n3luke@covab.mak.ac.ug
[1]Norwegian University of Life Sciences, Oslo, Norway
[2]Makerere University, Kampala, Uganda
Full list of author information is available at the end of the article

Although EVD is known to be very severe, there are some species of Ebola virus that cause less serious disease. For example, *Taï Forest ebolavirus,* formerly known as *Côte d'Ivoire ebolavirus*, has not been associated with any fatality and the only case ever reported recovered from the disease [6]. While there have been some reports of EVD being associated with a CFR of 100%, this CFR is attributed to only a single case fatality that did not result into transmission of the virus to other individuals [7, 8]. It seems that CFR differs from species to species, however, both *Ebola Sudan* and *Ebola Zaire* have shown a CFR of 100% [1]. Also, the CFR of the MVD outbreak that occurred in Uganda in 2014 was reported to be 100%, but again only one person was diagnosed and died from the disease [9]. The largest MVD outbreak was in Angola in 2004 with CFR of 90% [10] and in Democratic Republic of Congo (DRC) in 1998 with CFR of 83% [11].

There is evidence that a substantial proportion of infected humans in Central Africa seem to recover without being detected by the health care system, and apparently healthy individuals have been found to be seropositive for Ebola and Marburg viruses [12–15]. Furthermore, Marburg virus has been found in apparently healthy cave-dwelling fruit bats of species *rousettus aegyptiacus,* which are believed to be reservoirs for Marburg virus, and responsible for the spill over into human populations [16–19]. Because of the variations in the reported CFR and the presence of seropositive individuals, it is important to determine the severity and prevalence of these viral haemorrhagic fevers. This is important for forecasts and risk analysis especially during outbreaks for epidemic preparedness and response by affected countries. This will help to estimate how many infected people with EVD or MVD are likely to die from the disease during outbreaks. Whereas there are few studies that have estimated CFR of EVD [4, 5], these did not use a meta-analysis approach and no meta-analysis has been performed on CFR of EVD, MVD, seroprevalence of Ebola and Marburg viruses. Therefore, our aim was to determine the overall weighted estimate (effect size) of the CFR and seroprevalence of EVD and MVD using available published literature on outbreak reports, WHO and CDC databases and population based studies for seroprevalence of filoviruses (Marburg and Ebola viruses). We also explored whether CFR and seroprevalence of these filoviruses differs according to virus species and country.

Methods

Procedures for systematic reviews and meta-analysis have been developed to summarize scientific evidence from the literature. This work was done following the guidelines published in the PRISMA statement [20] and MOOSE guidelines for observational studies [21] as follows.

Literature search strategy

A detailed literature search was conducted by the authors in PubMed (as well as Medline), Web of Science and Google Scholar until 5th October 2015. In cases where there was no peer-reviewed publication for a known outbreak, data was retrieved from websites of WHO and CDC. The following key words were used; "ebola", "ebolavirus", "viral haemorrhagic fevers", "marburg virus disease", "marburg haemorrhagic fever", "marburg virus outbreak", "ebola virus disease outbreak", "marburg virus", "ebola outbreak", "seroprevalence of ebola virus", "seroprevalence of marburg virus" and "risk factors of viral haemorrhagic fevers". The search included all articles and outbreak reports about EVD and MVD and cross-referencing of primary articles was done to obtain the original articles. Since the number of outbreaks of EVD and MVD are known and few, efforts were made to obtain all information about these outbreaks from WHO and CDC websites and Ministries of health of respective countries.

Study selection criteria

Studies were included in the meta-analysis if they reported the total number of cases and total number of deaths from the outbreak of EVD or MVD. Also studies that were reporting CFR and sero-prevalence in percentages were included. Studies or reports that did not include total number of deaths or cases were excluded as well as studies that did not report original data (Fig. 1). We also excluded studies that reported outbreaks of Ebola species that are not pathogenic to humans and those species that have not caused mortality in humans. In cases where there were multiple publications, we used the one with the most complete data or the most recent one. In cases where there was controversy on the number of cases and deaths between studies, we cross-referenced with the respective ministries of health, WHO or CDC databases to reconcile these discrepancies. Seroprevalence studies included were only those that were population based and comprised apparently healthy individuals. We excluded articles that reported sero-prevalence during outbreaks or in sick individuals.

Data extraction

LN compiled a list of articles and discrepancies were discussed and resolved by consensus between FM, CK and JL. We used a standardized data extraction form and the following information was extracted for each qualifying study and outbreak report: i) author; ii) Country; iii) number of cases; iv) number of deaths; v) CFR (if reported); vi) month and year of outbreak; vii) year of publication viii)

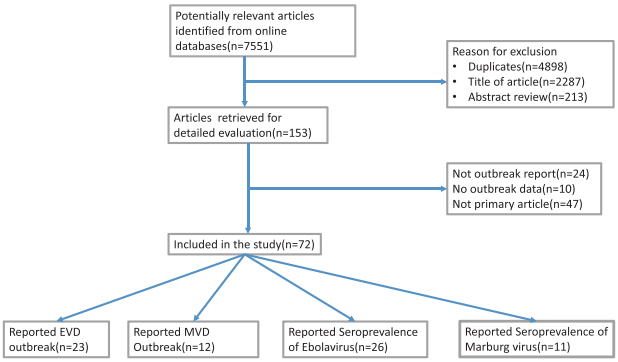

Fig. 1 Flow diagram for search strategy and article selection process from the literature databases

and species involved. For population-based seroprevalence studies, the following additional information was retrieved: i) sample size and ii) number of seropositive samples.

Statistical analysis

Data were collected in a Microsoft Excel® spreadsheet and outcome measures were calculated. CFR was calculated as number of deaths divided by reported cases whereas seroprevalence was calculated as number of individuals seropositive divided by total sample size in each study. Our effect size (ES), the principal summary measure, was the proportion represented by CFR and seroprevalence. We used the newly developed *metaprop* command [22] for performing meta-analysis of binomial data in STATA (StataCorp, College Station, TX, USA). The *metaprop* command was preferred to *metan* command because it implements procedures that are specific to binomial data and is appropriate for dealing with proportions close to or at the margins and also uses the Freeman-Tukey double arcsine transformations to stabilize the variances [22]. The meta-analysis of CFR was stratified by country and species where possible.

The following parameters were estimated: Cochran's Q indicating differences in true ESs, an estimate of the true variance of ESs between studies (our estimate of τ^2) and Higgins I^2 which is an estimate of what proportion of the observed variance that reflects real differences in ES. If I^2 is close to 0, then almost all the observed variation is spurious, and there is nothing to explain. If I^2 is large, then reasons for the observed variance should be evaluated [23, 24]. Sensitivity analysis was done by excluding studies that reported very few numbers or zero deaths or no seropositives. A meta-regression procedure was done to assess if factors such as species, country, year and month of outbreak influence CFR of both EVD and MVD using the traditional logit-transformation: Logit (prevalence) = ln [prevalence/ (1 – prevalence)] Variance (logit) =1/ (np) +1/[n (1 – p)] [25]. The Begg's and Egger's tests were used in combination with a funnel plot to assess potential publication bias and visualised using funnel plots [24, 26].

Results

Literature search result

Results from the literature search are illustrated in Fig. 1. The literature search yielded 7551 articles. Of these, 4898 were excluded as duplicates. After reviewing the titles and the abstract, only 153 articles were retrieved for detailed evaluation. After full evaluation of retrieved publications, 72 articles were included in this study. Of those included in the study, 23 reported outbreaks of EVD (Table 1) [3, 8, 27–41, 7, 42, 43], 12 reported outbreaks of MVD (Table 2) [10, 11, 42, 44–51], 26 reported seroprevalence of Ebola virus (Table 3) [8, 12–14, 28, 31, 52–54, 29, 55–70] and 11 reported sero-prevalence of Marburg virus (Table 4) [14, 15, 57, 61–64, 67, 71–73]. Most of the sero-prevalence studies reported both Marburg and Ebola viruses.

Two more outbreaks have occurred without human mortalities namely Ebola Reston [74, 75] and another caused by *Taï* Forest virus [6]. *Zaire ebolavirus* species was responsible for most of the outbreaks with 14/23 (60.9%) [8, 28, 30–32, 34–36, 39, 40, 41, 37, 76] followed by *Sudan ebolavirus* with 30.3% (7/23) outbreaks [27, 29, 38, 7, 42, 77] and lastly *Bundibugyo ebolavirus* 8.7% (2/23) [3, 42]. Most articles reported DRC (7/23) [8, 28, 32, 39, 40, 42, 76] and Uganda (5/23) [3, 33, 7, 42] as countries most affected by EVD outbreaks. Other countries reported include Gabon (4/23) [31, 34, 36, 78], Republic of Congo (3/23) [35, 37, 41], South Sudan (3/23) [27, 29, 38] and multiple countries in West Africa associated with the recent single outbreak [79–82]. Interestingly, most of the EVD outbreaks

Table 1 Summary of the studies included in a systematic review and meta-analysis describing case fatality rate for Ebola virus disease in Africa

Author and Year of Publication	Deaths	Cases	Country	Year and month of outbreak
WHO International Study Team, 1978 [27]	151	284	South Sudan	1976, June–November
International Commission, 1978 [28]	280	318	DRC	1976, Sept–Oct
Heymann et al., 1980 [8]	1	1	DRC	1977, June
Baron et al., 1983 [29]	22	34	South Sudan	1979, June–Oct
Amblard et al., 1997 [30]	30	49	Gabon	1994, November
Khan et al., 1999 [32]	255	315	DRC	1995, May
Georges et al., 1999 [31]	21	31	Gabon	1996, May
Milleliri et al., 2004 [34]	45	60	Gabon	1996, May
Okware et al., 2002 [33]	224	425	Uganda	2000, October
Nkoghe et al., 2005 [36]	97	124	Gabon	2000, December
Rouquet et al. (2005) [37]	128	143	ROC	2003, December
Boumandouki et al., 2005 [35]	29	35	ROC	2003, Oct–Dec
Onyango et al., 2007 [38]	7	17	South Sudan	2004, April–June
Nkoghe et al., 2011 [41]	10	12	ROC	2005, April–May
Leroy et al., 2009 [39]	186	264	DRC	2007, May and November
Wamala et al., 2010 [3]	39	116	Uganda	2007, August
Grard et al., 2011 [40]	15	32	DRC	2008, Jan
Shoemaker et al., 2012 [7]	1	1	Uganda	2011, May
Albariño et al., 2013 [42]	4	11	Uganda	2012, July
Albariño et al., 2013 [42]	3	6	Uganda	2012, Nov
Albariño et al., 2013 [42]	13	36	DRC	2012, August
Maganga et al., 2014 [43]	49	69	DRC	2014, July
WHO, 2016 [79, 90]	11323	28646	West Africa	March, 2014

DRC Democratic Republic of Congo, *ROC* Republic of Congo

Table 2 Summary of studies included in a systematic review and meta-analysis describing case fatality rate for Marburg virus from searched literature globally

Author and Year of Publication	Deaths	Cases	Country	Year & Month of outbreak
Siegert, 1972 [44, 45]	7	31	Germany and Yugoslavia	1967, August
Gear et al., 1975 [91]	1	3	Johannesburg, South Africa	1975, February
Smith et al., 1982 [92]	1	2	Kenya	1980, January
Johnson et al., 1996 [49]	1	1	Kenya	1987, August
Nikiforov et al., 1994 [48]	1	1	Russia	1990
Bausch et al., 2006 [11]	128	154	DRC	1998, October
Towner et al., 2006 [10]	227	252	Angola	2004, October
Adjemian et al., 2011 [51]	1	4	Uganda	2007, June
Centers for Disease & Prevention, 2009 [50]	0	1	USA from Uganda	2008, January
Timen et al., 2009 [93]	1	1	Netherlands from Uganda	2008, July
Albarino et al., 2013 [42, 94]	4	15	Uganda	2012, October
WHO, 2015 [95]	1	1	Uganda	2014, October

DRC Democratic Republic of Congo

Table 3 Summary of studies included in a systematic review and meta-analysis describing sero-prevalence of Ebola virus from literature

Author and Year of Publication	Sample size	Seropositive	Country
Van der Groen and Pattyn 1979 [96]	251	43	DRC
Saluzzo, Gonzalez et al. 1980 [97]	499	17	CAR
Bouree & Bergmann, 1983 [55]	1517	147	Cameroon
Johnson et al., 1983 [56]	741	8	Kenya
Van der Waals, Pomeroy et al. 1986 [57]	225	30	Liberia
Meunier et al., 1987 [58]	1528	319	CAR
Paix et al., 1988 [59]	375	4	Cameroon
Tomori, Fabiyi et al. 1988 [60]	1,677	30	Nigeria
Gonzalez et al., 1989 [72]	5070	629	Central Africa
Mathiot, Fontenille et al. 1989 [61]	381	17	Madagascar
Johnson, Gonzalez et al.1993a [63]	427	75	CAR
Johnson, Gonzalez et al. 1993b [64]	4295	914	CAR
Busico et al., 1999 [66]	575	24	DRC
Nakounne, Selekon et al. 2000 [67]	1762	104	CAR
Heffernan et al., 2005 [69]	979	14	Gabon
Allela et al., 2005 [68]	439	64	Gabon
Lahm, Kombila et al. 2007 [70]	1147	14	Gabon
Becquart et al., 2010 [12]	4349	665	DRC
Heymann et al., 1980 [8]	1096	79	DRC
Burke et al., 1978 [28]	984	38	DRC
Baron et al., 1983 [29]	106	23	Sudan
Georges et al., 1999 [31]	441	58	Gabon
Becker, Feldmann et al. 1992 [62]	1288	11	Germany
Gonzalez, Nakoune et al. 2000 [14]	1331	71	CAR
Bertherat, Renaut et al. 1999 [65]	236	24	Gabon
Nkoghe, Padilla et al. 2011 [13]	4349	667	DRC

DRC Democratic Republic of Congo, *ROC* Republic of Congo, *CAR* Central African Republic

Table 4 Summary of studies included in a systematic review and meta-analysis describing sero-prevalence of Marburg disease from published literature

Author and Year of Publication	Sample size	Seropositive	Country
Van der Waals, Pomeroy et al. 1986 [57]	225	3	Liberia
Gonzalez, Josse et al. 1989 [72]	5070	20	Central African countries
Johnson, Ocheng et al. 1983 [71]	1899	8	Kenya
Mathiot, Fontenille et al. 1989) [61]	384	0	Madagascar
Becker, Feldmann et al. 1992 [62]	1288	34	Germany
Johnson, Gonzalez et al. 199a [63]	427	5	CAR
Johnson, Gonzalez et al. 1993b [64]	4295	137	CAR
Gonzalez, Nakoune et al. 2000 [14]	1340	33	CAR
Nakounne, Selekon et al. 2000 [67]	1762	35	CAR
Bausch, Borchert et al. 2003 [15]	912	15	DRC
Borchert, Mulangu et al. 2006 [73]	300	0	DRC

DRC Democratic Republic of Congo, *CAR* Central African Republic

occurred during months of May, June and July and no outbreaks were reported in the month of February.

Meta-analysis and meta-regression of CFR and seroprevalence of EVD

The weighted CFR of EVD from 23 outbreaks was 65% (95% CI: 54–76%) (Fig. 2). There was a substantial between-study variance indicating heterogeneity in the overall CFR of EVD, $I^2 = 97.98\%$. On stratification by Ebola virus species, the CFR for *Sudan ebolavirus* was 53%, *Bundibugyo ebolavirus* was 34%, whereas that of *Zaire ebolavirus* was 75%. From the meta-regression, the CFR for *Zaire ebolavirus* was higher compared to other Ebola species (=0.006, Coefficient = 0.19, 95% CI = 0.063 - 0.588). In sub-analysis analysis by country, the highest CFR for EVD was observed in Republic of Congo (89.0%, 84.0–93.0%) whereas the lowest was found in Uganda (43.0%, 27.0–61.0%) (Fig. 3). However, the large West African EVD outbreak that affected multiple countries had an even lower CFR at 40% (39–40%). The pooled ES for Ebola virus seroprevalence was 8% [5–11%) with substantial between-study variance ($I^2 = 98.7\%$) (Fig. 4).

Meta-analysis and meta-regression of CFR and seroprevalence of MVD

The MVD CFR was lower than that of EVD (61%) (Fig. 5). There was no significant difference between CFR of MVD and different variables in the meta-regression model ($P = 0.637$). The pooled seroprevalence of Marburg virus was lower than that of Ebola virus at 1.2% (0.5–2%) (Fig. 6).

Publication bias

In the funnel plots, asymmetry was evident which gives rise to suspected publication bias (Fig. 7). Egger's test

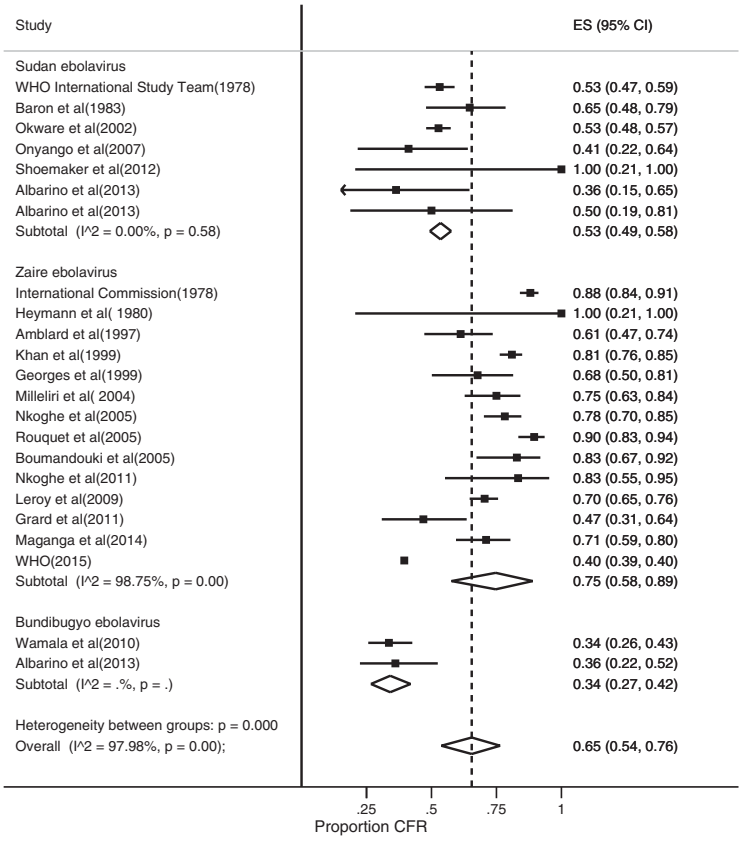

Fig. 2 Forest plot showing stratified meta-analysis of CFR of Ebola Virus Disease by virus species estimated by the random effects model (I^2 = Higgins statistic, ES = Effect size, CI = Confidence Interval)

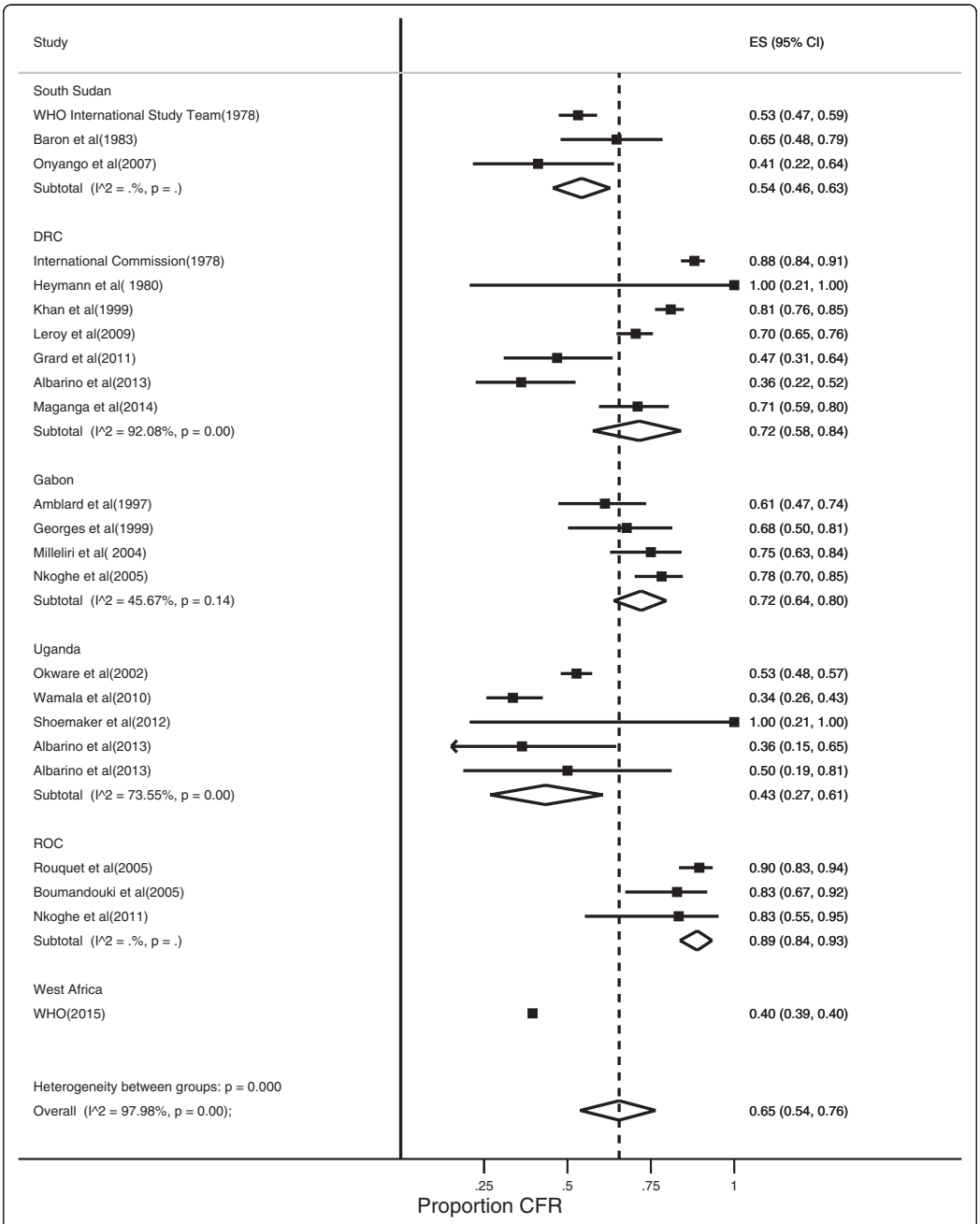

Fig. 3 Forest plot showing stratified meta-analysis of CFR of Ebola virus disease by country estimated by the random effects model (I^2 = Higgins statistic, ES = Effect size, CI = Confidence Interval, DRC = Democratic Republic of Congo, ROC = Republic of Congo)

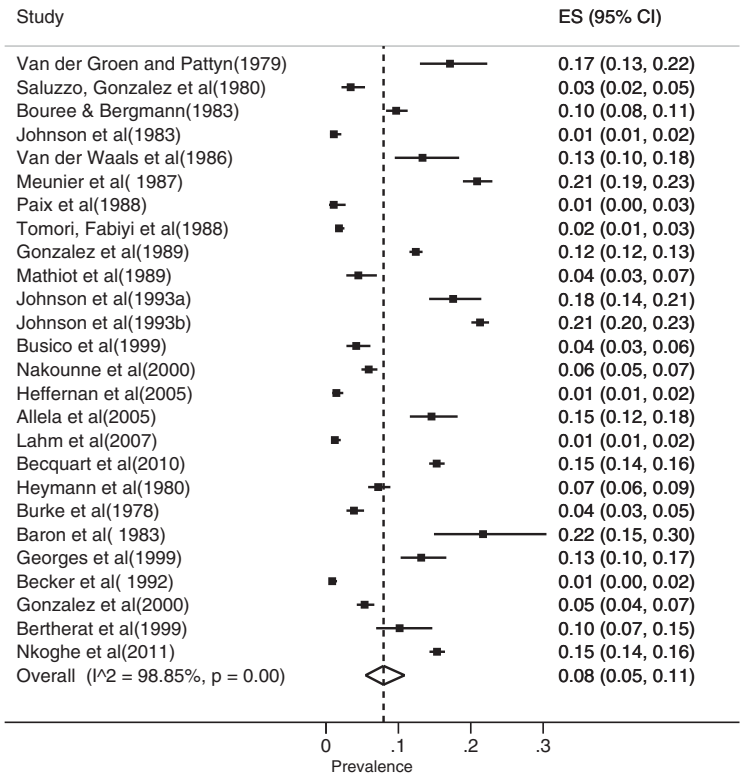

Fig. 4 Forest plot for the meta-analysis of sero-prevalence studies of Ebola virus (I^2 = Higgins statistic, ES = Effect size, CI = Confidence Interval)

was significant for studies reporting CFR and seroprevalence of EVD and MVD ($P = 0.001$, $P < 0.001$, $p = 0.032$, and 0.046 respectively). However, the Begg's bias test was not significant for studies reporting CFR of EVD and MVD ($p = 0.091$ and $p = 0.293$ respectively), seroprevalence of MVD ($p = 0.95$), but was significant for studies reporting seroprevalence of EVD ($p = 0.007$).

Discussion

Our findings show that the overall pooled CFR of EVD of 65% was lower than the previously reported CFR of 90% [83]. This indicates, despite substantial heterogeneity, that more than half of the individuals who contract EVD are more likely to die. Although this CFR appears to be high, it is lower than the exaggerated figure of 90%. This high CFR tends to cause fear and panic in the general public and hence interferes with response mechanisms [84]. The CFR in our study is similar to that reported by Lefebvre *et al.* [4], who reported a CFR of 65% in a study done using WHO database on EVD outbreaks. Although there have been cases of EVD and MVD with 100% CFR [8, 7], these were isolated single cases that should not be generalized by scientific community to consider Ebola and Marburg viruses as highly virulent diseases with CFR of up to 90%. There have been reports with a higher CFR than our maximum of 76% [28, 35, 37, 41], but these either happened long time ago [28] where there was little knowledge about the disease or happened in very remote places where health care delivery systems are not robust.

The high CFR of EVD in Republic of Congo (89%) compared to Uganda (43%) may be due to partly, differences in health care system and response mechanisms to outbreaks, but also the severity of the species of Ebola virus involved. For example, Uganda has developed a robust surveillance system for detecting these viral haemorrhagic fevers and epidemic response is started within hours of a positive diagnosis at a CDC supported laboratory in the country [85]. The well-established disease surveillance system and organised health care

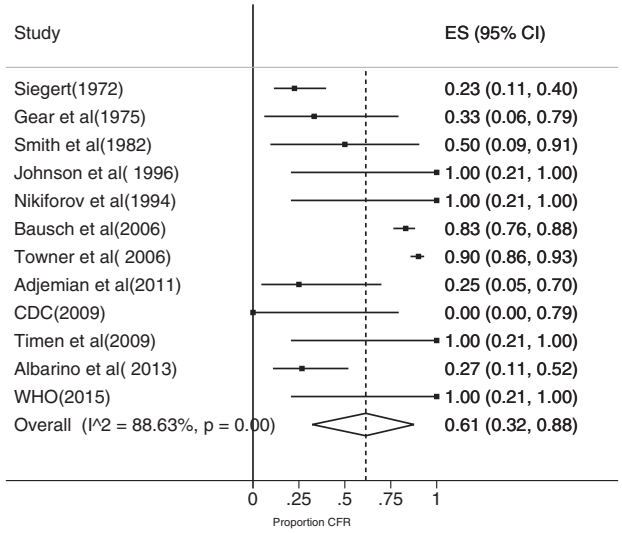

Fig. 5 Forest plot for a meta-analysis of CFR of Marburg virus disease estimated using a random effects model (I^2 = Higgins statistic, ES = Effect size, CI = Confidence Interval)

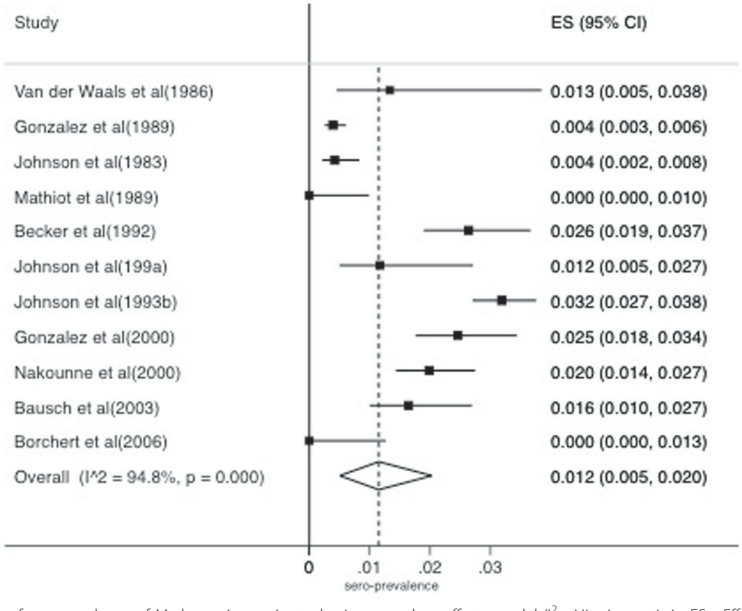

Fig. 6 Meta-analysis of seroprevalence of Marburg virus estimated using a random effects model (I^2 = Higgins statistic, ES = Effect size, CI = Confidence Interval)

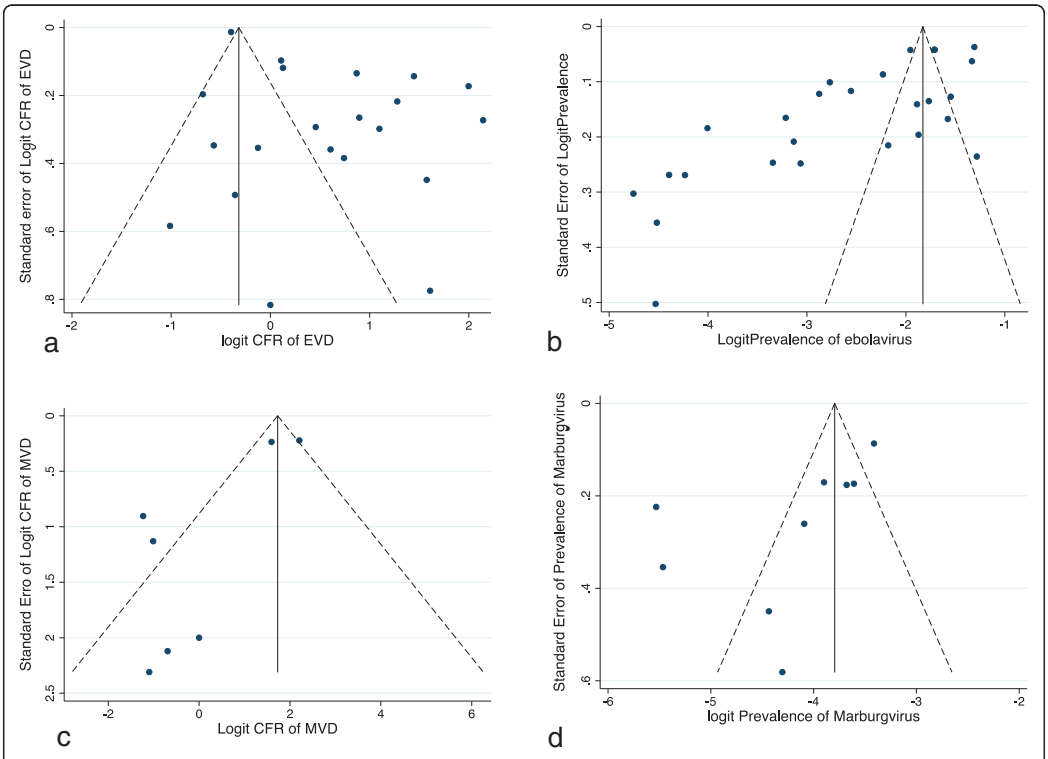

Fig. 7 Funnel plots assessing publication bias in studies reporting case fatality rate and seroprevalence of Ebola virus disease and Marburg virus disease. **a** Funnel plot of the point estimates of the logit CFR of EVD, **b** Funnel plot of the point estimates of the logit prevalence of EVD, **c** Funnel plot of the point estimates of the logit CFR of MVD, **d** Funnel plot of the point estimates of the logit prevalence of MVD

delivery in endemic areas might explain the lower CFR for EVD observed in Uganda. But it is also important to note that Uganda has been affected by the less pathogenic species of Ebola virus (*Sudan ebolavirus* and *Bundibugyo ebolavirus*) as compared to DRC and West African countries that have experienced *Zaire ebolavirus* Also, it is important to look at the denominators and numerators when interpreting the CFR. In this analysis, we see that CFR of EVD in a large outbreak in West Africa that affected multiple countries is at CFR of 40% using WHO data, but this alone would be misleading if the real numbers of deaths and cases were not looked at. As of 30th March 2016, there were 11323 deaths and 28646 cases due to EVD from all countries affected by that outbreak.

Another significant finding of our study was the variation in the severity and CFR among the pathogenic species of Ebola virus. Zaire *ebolavirus* (CFR, 75%) was found to be the most severe followed by *Sudan ebolavirus* (CFR, 53%), while *Bundibugyo ebolavirius* (CFR, 34%) was the least severe species. This finding is supported by McCormick *et al.*, who described differences in severity and filovirus dynamics [86, 87]. The reasons for severity of *Zaire ebolavirus* are unclear, thus there is a need for further research to determine whether genetic differences are responsible for the variation in pathogenesis of these species. There was also heterogeneity within *Zaire ebolavirus* outbreaks ($P < 0.001$) meaning that these outbreaks, although caused by the same species are not always similar. The heterogeneity could further be explained by differences in outbreak investigation designs or approaches, location of the outbreak and data collection methods. This is further supported by the strains that have been found within Ebola Zaire species [40]. There was less heterogeneity in outbreak reports for *Bundibugyo ebolavirus* and *Sudan ebolavirus* probably due to few outbreaks that have been caused by these species. However, the meta-regression did not show any influence on CFR of EVD by country of outbreak ($p = 0.249$). This is probably due to low power given the few number of outbreaks that we have had globally.

With the *Metaprop* command for meta-analysis of marginal proportions [22], it was possible to estimate the 95% confidence intervals for MVD as 61% (32–88%). The CI was very wide because of the few outbreaks and the number of cases involved in MVD outbreaks as compared to EVD outbreaks. Dropping studies with 100% or 0% CFR for MVD, the CFR reduced from 61 to 53%. With few outbreaks of Marburg virus in different countries, there is a high variation that would impact the estimation of CFR for MVD, but this was not significant from the meta-regression ($p = 0.913$).

We found that apparently healthy individuals in central African countries, that are endemic for viral haemorrhagic fevers, had a 5 and 1% chance of having antibodies against Ebola and Marburg viruses, respectively. This finding suggests that some individuals who get infected with filoviruses make a full recovery without severe complications and being documented by healthcare systems. Although the sero-prevalence is low, it is important that these seropositive individuals are detected early enough because of greater mortality and socio-economic implications associated with these infections. Because serological tests have been reported to have low specificity and there is a lot of cross-reactivity of filoviruses with other viral haemorrhagic fevers [88], this finding should be interpreted with caution. It is important that specific and more accurate tests are developed to accurately measure antibody response against filoviruses and progress in this direction has been made due to the recently approved rapid diagnostic test for Ebola virus by WHO [89].

The limitation of our ES estimates was the heterogeneity that was observed between studies. Efforts to identify sources of heterogeneity were made, and many unmeasured factors could have influenced CFR during outbreaks. These reports had data that were collected using different methods and hence combining them to produce one effect was likely to produce high heterogeneity. Sensitivity analysis by dropping single cases with 100% mortality did not have substantial impact on the result. Funnel plots and Beggs tests suggested that publication bias might have been present, meaning that studies with negative results about Ebola and Marburg viruses are less likely to be published hence affecting the estimate of seroprevalence and CFR for EVD and MVD.

The fact that laboratory tests for Ebola and Marburg viruses are expensive, used only in specific laboratories and that serological tests are not specific might influence the publication of studies done with these tests.

Conclusions

The CFR for Ebola and Marburg viruses is still moderately high but not as high as has been reported in the media and other publications. The CFR of EVD and MVD is higher in countries with poor disease surveillance systems. This calls for an improved surveillance system that will enhance early detection and response to these filovirus outbreaks to avoid a pandemic. The presence of seropositive individuals in apparently health populations indicate that cases go undetected by the health care system in affected countries; further calling for robust surveillance for Ebola and Marburg viruses.

Abbreviations

CDC: Centres for disease control and prevention USA; CFR: Case fatality rate; CI: Confidence interval; DRC: Democratic Republic of Congo; EVD: Ebola virus disease; MVD: Marburg virus disease; ROC: Republic of Congo; WHO: World Health Organization

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Availability of data and materials

The dataset supporting the findings in this meta-analysis is included in the article from Tables 1, 2, 3 and 4.

Authors' contributions

Conceived and designed the protocol: LN, ES, CK. Execution of search strategy and sifting: LN, JL, BM, MF, RK. Manuscript preparation: LN, CK, RK, BM, MF, JL and ES. All authors read and approved the final manuscript.

Authors' information

LN is an Epidemiologist with a background in Veterinary Medicine. He has been working as a zoonotic disease Epidemiologist especially focussing of Ebola and Marburg virus outbreaks in Uganda for the last five years. He is currently pursuing a PhD in Epidemiology of Ebola and Marburg viruses in Uganda at the Norwegian University of Life Sciences, Oslo Norway.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Author details

[1]Norwegian University of Life Sciences, Oslo, Norway. [2]Makerere University, Kampala, Uganda. [3]Norwegian Medicines Agency, Oslo, Norway. [4]Ulm University, Ulm, Germany. [5]Uganda Virus Research Institute, Entebbe, Uganda.

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PAPER II

PLOS | CURRENTS OUTBREAKS

Ecological Niche Modeling for Filoviruses: A Risk Map for Ebola and Marburg Virus Disease Outbreaks in Uganda

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Citation

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Authors

Luke Nyakarahuka

1) Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Oslo, Norway; 2) Department of Biosecurity, Ecosystems and Veterinary Public Health, Makerere University, Kampala Uganda; 3) Department of Arbovirology, Emerging and Re-Emerging disease, Uganda Virus Research Institute, Entebbe, Uganda.

Samuel Ayebare

Climate Change and Biodiversity Unit, Wildlife Conservation Society, Bronx, New York, United States of America.

Gladys Mosomtai

Earth Observation Unit, International Centre for Insect Physiology and Ecology, Nairobi, Kenya.

Clovice Kankya

Department of Biosecurity, Ecosystems and Veterinary Public Health, Makerere University, Kampala, Uganda.

Julius Lutwama

Department of Arbovirology, Emerging and Re-Emerging diseases, Uganda Virus Research Institute, Entebbe, Uganda.

Frank Norbert Mwiine

Department of Biomolecular Resources and Biolab Sciences, Makerere University, Kampala, Uganda.

Eystein Skjerve

Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Oslo, Norway.

Abstract

Introduction: Uganda has reported eight outbreaks caused by filoviruses between 2000 to 2016, more than any other country in the world. We used species distribution modeling to predict where filovirus outbreaks are likely to occur in Uganda to help in epidemic preparedness and surveillance.

Methods: The MaxEnt software, a machine learning modeling approach that uses presence-only data was used to establish filovirus – environmental relationships. Presence-only data for filovirus outbreaks were collected from the field and online sources. Environmental covariates from Africlim that have been downscaled to a nominal resolution of 1km x 1km were used. The final model gave the relative probability of the presence of

filoviruses in the study area obtained from an average of 100 bootstrap runs. Model evaluation was carried out using Receiver Operating Characteristic (ROC) plots. Maps were created using ArcGIS 10.3 mapping software.

Results: We showed that bats as potential reservoirs of filoviruses are distributed all over Uganda. Potential outbreak areas for Ebola and Marburg virus disease were predicted in West, Southwest and Central parts of Uganda, which corresponds to bat distribution and previous filovirus outbreaks areas. Additionally, the models predicted the Eastern Uganda region and other areas that have not reported outbreaks before to be potential outbreak hotspots. Rainfall variables were the most important in influencing model prediction compared to temperature variables.

Conclusions: Despite the limitations in the prediction model due to lack of adequate sample records for outbreaks, especially for the Marburg cases, the models provided risk maps to the Uganda surveillance system on filovirus outbreaks. The risk maps will aid in identifying areas to focus the filovirus surveillance for early detection and responses hence curtailing a pandemic. The results from this study also confirm previous findings that suggest that filoviruses are mainly limited by the amount of rainfall received in an area.

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Introduction

Uganda has experienced eight filovirus outbreaks; five Ebola Virus Disease (EVD) and three Marburg virus disease (MVD), between 2000 and 2016, more than any other country in the world.

The first outbreak in Uganda was caused by Ebolavirus of the species Sudan ebolavirus in 2000 in the Northern district of Gulu, where 425 cases were registered with a case fatality rate (CFR) of 53%[1]. The second outbreak was caused by Bundibugyo Ebolavirus in the western part of Uganda bordering with Democratic Republic of Congo (DRC), with 192 cases and a CFR of 34%[2,3] . In 2011, another EVD outbreak occurred where only one case was involved in Luweero district Zirobwe village, 45 km North of Uganda's Capital City Kampala[4]. Two more EVD outbreaks were observed in 2012, one in June in the Western District of Kibale and another in November, Luweero district in Central Uganda[5].

Likewise, three outbreaks of MVD have occurred in Uganda; the first one was in Kamwenge district in 2007 associated with mining activity in the Kitaka gold mine that is occupied by bats[6]. This outbreak was later linked to cave-dwelling Egyptian fruit bats (*Rousettus aegyptiacus*) that occupy these mines, as they tested positive for Marburg virus by polymerase chain reaction (PCR)[7,8]. Another outbreak of MVD was in 2012 where several districts were involved with a CFR of 58% (15/26)[9]. This outbreak was also traced back to the same gold mines in Western Uganda, and subsequent testing of the bats in the mines revealed a spill over to human populations[10]. The latest MVD outbreak was in Kampala where the only fatal case was a health worker, and no other cases were identified[11].

It is hypothesized that distribution of filoviruses is limited by the distribution of the bats, which are known probable reservoirs. All the filovirus outbreaks in humans have been reported to originate from Sub-Sahara Africa and only one species, Reston virus that is not known to infect humans was detected outside Sub-Sahara Africa in The Philippines[12]. It has been suggested that transmission from the natural reservoir occurs when humans get into contact with the reservoir or its body fluids such as feces, urine, and blood via activities such as hunting and consumption of bush meat[13]. Because previous outbreaks in Central Africa have been linked to reports of bush meat consumptions and deaths of wildlife[14], many hypotheses have been put forward to suggest wildlife such as bats, primates, and antelopes as possible sources of infection. The debate on bats as potential reservoirs of Ebolaviruses is still not concluded, as no *Ebolavirus* has been isolated from bats despite

finding some bats seropositive for Ebolavirus and others with viral RNA[15]. The role of non-human primates as reservoirs has been unconvincing since they do die from infection with filoviruses[16,17,18,19]. Other wildlife that has been reported to be infected by *Ebolavirus* was one duiker, whose bone tested positive by PCR in Republic of Congo bordering Gabon[19]. Dogs and pigs are the only domestic animals associated with ebolaviruses. Dogs were found to be IgG seropositive in Gabon[20] whereas Reston virus has been reported in pigs and have shown potential for infection with Ebola virus[21,22,23]. Unlike EVD, there is progress in research in trying to describe the reservoirs of Marburg virus. Bats of species *Rousettus aegyptiacus*, found in Kitaka gold mine and Python cave from the Albertine region in Western Uganda have been described as potential reservoirs of Marburg virus in Uganda[8,10,24]. The bats in these caves have been linked to three MVD outbreaks, where artisanal gold miners got infected with Marburg virus[6,9]. Transmission of Marburg virus in human populations just like Ebolaviruses happens after a spillover event from the natural reservoir in wildlife. Lack of a clear reservoir and true source of infection or spill-overs into human populations has been a call for alternative methods of heightening surveillance and developing risk maps is one of them.

Situated in the rich and complex ecological systems with high biodiversity in East Africa, Uganda is not only affected directly by filovirus outbreaks but also vulnerable to outbreaks from neighboring countries such as DRC. For epidemic preparedness and response, Uganda's health surveillance system needs to know where and when these epidemics are likely to occur. This will allow them to conduct active surveillance focusing in those areas for early detection to avoid pandemics and also focus research on reservoirs. This can be achieved by applying spatial epidemiology modeling techniques. One such technique is Ecological Niche Modeling (ENM) also known as Species Distribution Modeling (SDM), that has been used to establish the relationship between species and their environment[25,26,27,28]. ENM has also been used to predict the ecology and distribution of filoviruses before. Peterson *et al* (2014) used a Genetic Algorithm for Rule-Set Production (GARP) model to predict suitable environments for filoviruses as being in afro-tropics where EVD was being predicted more in the humid rain forest of Central and West Africa while MVD was more predicted to occur in the drier and more open areas of Central and East Africa[29]. More efforts were made to improve the spatial prediction model for MVD for Africa using a Bioclimatic variable (Bioclim)[30], which predicted filoviruses mainly in Zimbabwe and abroad potential distribution across the arid woodland regions of Africa[31]. Furthermore, Pigott *et al* (2014) developed zoonotic niche maps for Marburg and Ebola viruses in Africa using species distribution models[32,33]. In these maps, they have predicted EVD at risk areas occupied by 22 million people while MVD is predicted to occur in 27 countries across Sub-Sahara Africa. Enhanced vegetation index which corresponds to high levels of rainfall was identified as the most important variable limiting the distribution of the Ebola virus in Africa[32,33].

These predictions are not country specific, and they lack details of individual countries regarding vector and raster data. For example, they used online databases that are not accurate especially in estimating environmental covariates and getting coordinates of index cases, hence, affected countries find these maps limited for focused and targeted surveillance

A Maximum Entropy species distribution modeling environment (MaxEnt) has been used to predict the ecological niche for various species. The MaxEnt algorithm uses presence-only occurrence records to estimate the actual or potential geographic distribution of a species[34] and has been known to outperform other species' distribution modeling approaches such as Domain, Generalized Additive Models (GAM), Generalized Linear Models, Genetic Algorithm (GARP) and Bioclim[35].

MaxEnt models have been used widely to predict ecological niches of different vectors and disease-causing organisms[36,37,38,39,40,41,42,43], but it has not been used for prediction of filovirus outbreaks in Uganda. Briefly, MaxEnt is a multipurpose machine-learning technique and aims at estimating the probability of distribution of a species occurrence using the environmental features. Our major aim was to develop a country-specific risk map for Uganda using updated data on EVD/ MVD outbreaks and bat occurrence and environmental variables specific for Uganda using the MaxEnt modeling approach. The model outputs will improve filovirus epidemic preparedness, surveillance and response, and in the search for a reservoir especially in a disease prone country like Uganda

Materials and methods

EVD, MVD and Bat occurrence data

A total of 16 locations of the Ebolavirus outbreaks in Uganda since 2000 was obtained from published databases[44]. An additional 27 occurrence points for Ebola and Marburg virus diseases outbreaks were collected from the field where these outbreaks occurred especially for new outbreaks whose locations were not collected before. All locations where confirmed cases of Marburg or Ebola viruses were reported were collected with Global Positioning System (GPS) receiver and points were entered into an Excel spreadsheet. A total of 43 filovirus outbreak occurrence points (30 for EVD outbreak and 13 for MVD outbreak) were used for this prediction model (Supporting Information S1 File; see Appendix). These filovirus occurrence points represent households in villages where confirmed cases were residing. Due to the contagious nature of filoviruses, one household had more than one cases hence the reason for not using all the 562 EVD cases and 20 MVD cases. A fruit bat location survey was also done to determine the location of fruit bats in a cross-section of Uganda. We purposively selected districts to scout for bats based on previous filovirus outbreaks and anecdotal reports of bats in trees. Using a snowballing approach, we collected 84 fruit bat locations using a GPS receiver from different districts of the country. Here community members acted as informers of the roosting locations of fruit bats and caves that contain bats.

An additional, 517 bat locations from all over Uganda were generously provided by Kityo Robert (Department of Zoology, Makerere University Kampala Uganda) also published in Uganda Bat Atlas[45], resulting in a total of 601 bat coordinates (Supporting Information S1 File; see Appendix).

Environmental covariates

Ecologically suitable environmental covariates for filovirus outbreaks for Uganda were compiled from Africlim[46], with a spatial resolution of 1 km. The environmental covariates considered were moisture (mean annual rainfall, rainfall wettest month, rainfall driest month, rainfall seasonality, rainfall wettest quarter, rainfall driest quarter, annual moisture index, moisture index arid quarter, number of dry months, length of longest dry season) and temperature variables (mean annual temperature, mean diurnal range in temperature, isothermality, temperature seasonality, maximum temperature warmest month, minimum temperature coolest month, annual temperature range, mean temperature warmest quarter, mean temperature coolest quarter, potential evapotranspiration). We used ENMTOOLs; a toolbox that facilitates quantitative comparisons of environmental niche models[47] to test for multicollinearity between the predictor variables and we ran a pairwise Pearson correlation, and only variables with less than (+/-0.75) correlation were retained in the final prediction model (Supporting Information S2 File; see Appendix). After this test, only seven environmental variables were retained (Table 1); three moisture variables (Rainfall seasonality, Rainfall driest quarter, and mean annual rainfall) and four temperature variables (Temperature seasonality, Mean diurnal range in temperature, mean annual temperature and Isothermality).

Ecological Niche Model

We used MaxEnt Version 3.3k for modeling distribution of filovirus using default settings (Auto features, convergence threshold=0.00001, the maximum number of background points=10,000, regularization multiplier=1). A logistic probability map was generated showing the relative probability of the presence of filoviruses survival on a scale ranging between 0 and 1[48]. The occurrence data was subdivided into k-folds where 25% was set aside for testing the accuracy of the model, whereas 75% was used for training the model. However, there were few presence records (10) for the Marburg cases therefore, all the records were used in training the model. The Receiver Operating Curve (ROC) was used to assess the overall model predictive performance, a measure of the ability of the model to distinguish presence from absence of a species with a value of 1 indicating a perfect prediction while 0.5 is as good as a random prediction[49,50]. A jackknife test was used to evaluate individual covariate importance in the model developments (Supporting Information S3 File; see Appendix). To improve model robustness, 100 replicates were averaged for the final model outputs. MaxEnt outputs were imported into ArcGIS 10.3 mapping software to develop final maps.

Bioclim Nomenclature	Variable	Units	Variable Description
Moisture Variables			
BIO15	Rainfall seasonality	mm	Standard deviation over monthly values
BIO17	Rainfall driest quarter	mm	Any consecutive 3-month period
BIO12	Mean annual rainfall	mm	Sum of Monthly Rainfall
Temperature Variables			
BIO4	Temperature seasonality	°C	Standard deviation over monthly values
BIO2	Mean diurnal range temperature	°C	Mean of monthly (Max-min temp)
BIO1	Mean annual temperature	°C	Mean of monthly means
BIO3	Isothermality	°C	100X(Mean diurnal range temperature/Annual Tem Range)

Table 1: Environmental variables used in the models

Results

The bat occurrence and filovirus outbreak locations

As shown in Figure 1, bats are distributed all over Uganda, with a high distribution around water bodies which is a core need for survival. Areas around Lake Victoria, River Nile, and Western Rift Valley have high numbers of bats. Their locality is in line with regions that have reported filovirus outbreaks in Uganda.

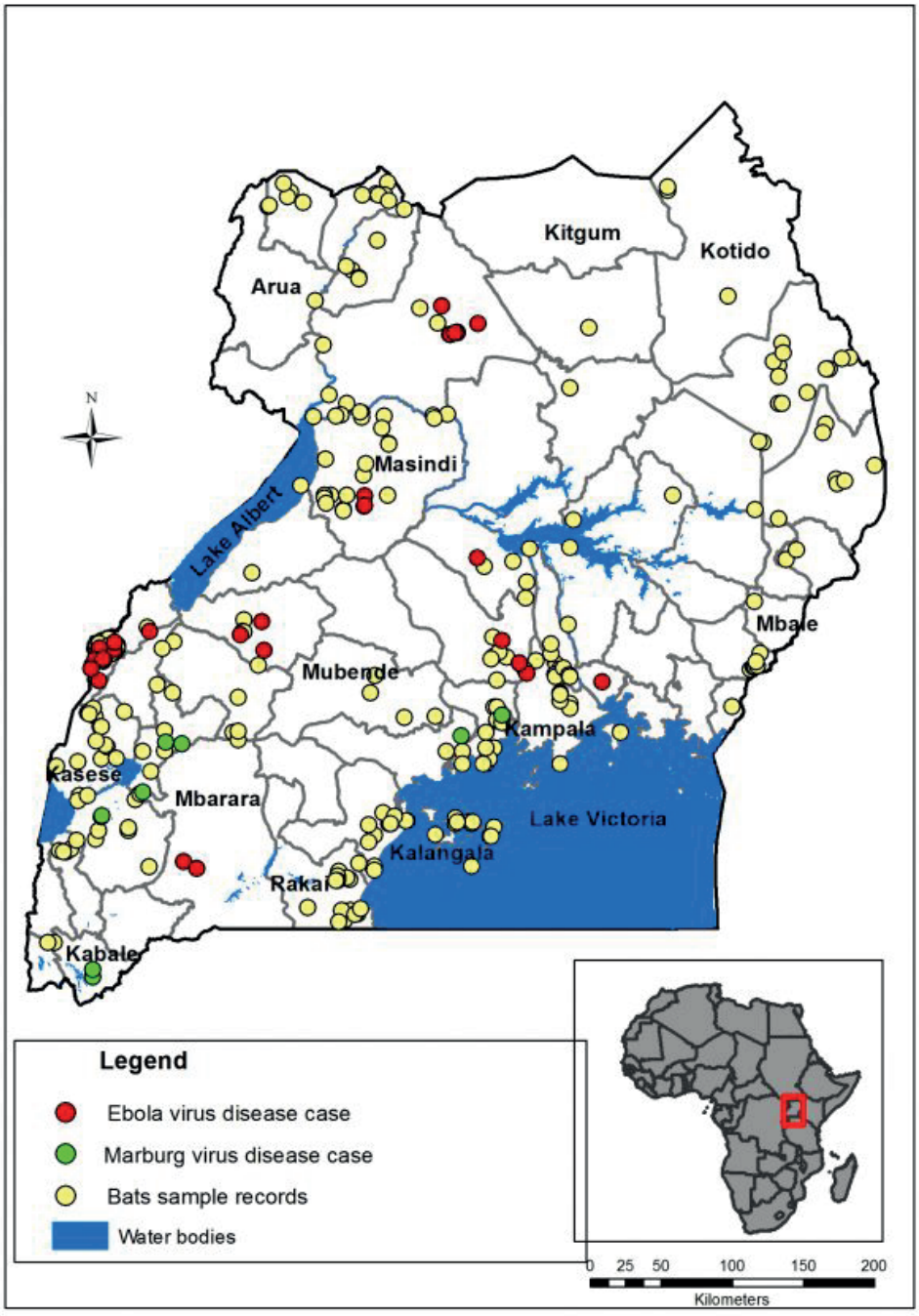

Fig. 1: Map of Uganda showing outbreak locations of Ebola and Marburg virus diseases and bat locations included in the Maxent modeling Environment

Bat distribution in Uganda

From 100 bootstrap replicates, a bat distribution map was generated (mean AUC=0.80; SD=0.012). Compared to a random prediction of AUC 0.5, our model was able to distinguish presence from the absence of bats within the geographic space with a high accuracy[51]. The relative probability of presence (RPP) ranged from highly suitable areas represented by red to orange colors to unsuitable areas represented by the green color in Figure 2A. The map shows that most areas in Uganda are suitable habitats for bats (both insect and fruit bats) with high RPP occurring in the following districts; Mbarara, Bushenyi, Bundibugyo and Kabale located in the western part of Uganda, around Lake Victoria (Kampala and Luweero districts) and in eastern region of Mbale and Soroti districts. Moderately suitable regions largely cover most parts of Uganda. The RPP of bats were mainly influenced by rainfall driest quarter with 24.7%, mean annual rainfall with 17.2%, mean diurnal range in temperature with 14.5%, and isothermality with 11.5% (Table 2).

Ebola virus distribution

High RPP for EVD outbreak was predicted in more than half of the country with hotspots in Western Rift valley districts of Bundibugyo, Masindi, Kibale and Hoima, Kasese, Kabarole, Kamwenge, Bushenyi and Ibanda as shown in Figure 2B (mean AUC=0.90; SD=0.024). In Central Uganda, Luweero, Kayunga, Mpigi, Kampala, Mityana and Nakasongola districts are predicted as potential areas for EVD outbreaks. In the eastern part of the country, it is mainly the Busoga region along River Nile and Mbale district around Mt. Elgon that are potential EVD hot spots. Other places that have not recorded outbreaks before but are predicted as potential probable areas for the spread of EVD include areas surrounding Lake Victoria and around Mount Elgon. A low RPP for EVD outbreak was predicted in North Eastern Uganda (Karamoja region) and Northern Uganda in the districts of Kitgum and Pader. Rainfall seasonality (33.2%), Mean annual rainfall (22.7%), rainfall of the driest quarter(20.8%) and mean diurnal range in Temperature (9.9%) had the highest relative contribution in predicting Ebola virus ecological suitability (Table 2).

Marburg virus disribution

The map in Figure 2C shows that Western, Southwestern and Central Uganda are potential areas for outbreaks of Marburg cases(AUC=0.92). Unlike predicted potential areas for EVD, predicted areas for MVD are mainly in the western sub-regions of Ankole, Tooro, Bunyoro, and Rwenzori region extending into DRC. Areas in the North and Eastern part of Uganda have a low or no relative probability of presence for MVD outbreaks as shown by the green color in Figure 2C. Temperature seasonality (68.2%) and rainfall seasonality (25.3%) contributed heavily to the model prediction (Table 2). Notably, temperature seasonality had the highest influence in MVD model compared to other variable contributions in all the models. However, the occurrence points were few in number to give us an accurate prediction.

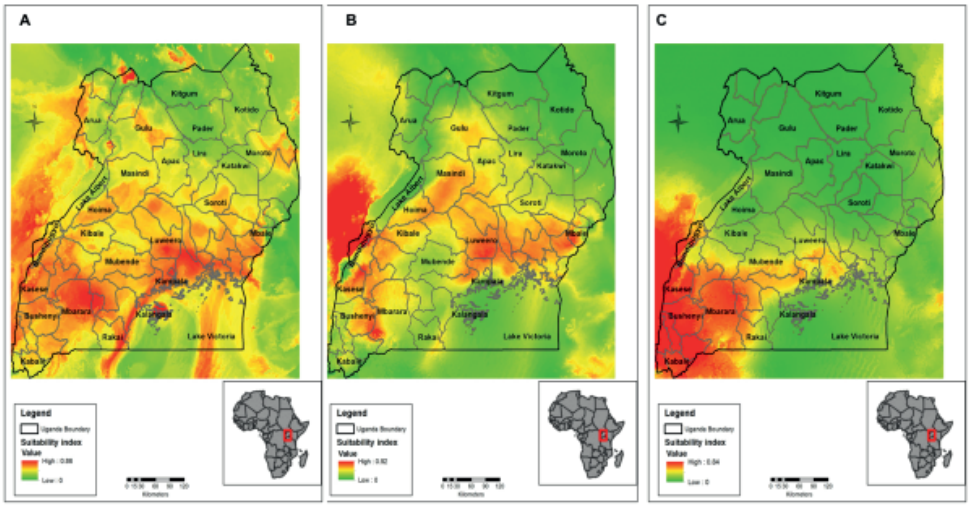

Fig. 2: Maps showing bats, EVD and MVD distribution in Uganda with high Relative Probability Presence represented in red while low in green.

A: Relative probability of presence of bats, hypothesized as reservoirs of filoviruses (AUC=0.80), B: Relative probability of presence of Ebola Virus disease outbreak (AUC=0.90), C: Relative probability of presence of Marburg Virus disease outbreak (AUC=0.92.

Filovirus distribution

Combining Marburg and Ebola virus occurrence points (Figure 3), we see the range of the possible distribution of filovirus, mainly in western, southwestern Uganda, Victoria basin districts and eastern Uganda (mean AUC=0.90; SD =0.023). Predictor variables that contributed more than 75% in the model include; rainfall seasonality (29.6%), rainfall of the driest quarter (26.3%), Temperature seasonality and mean annual rainfall (14.9%) (Table 2).

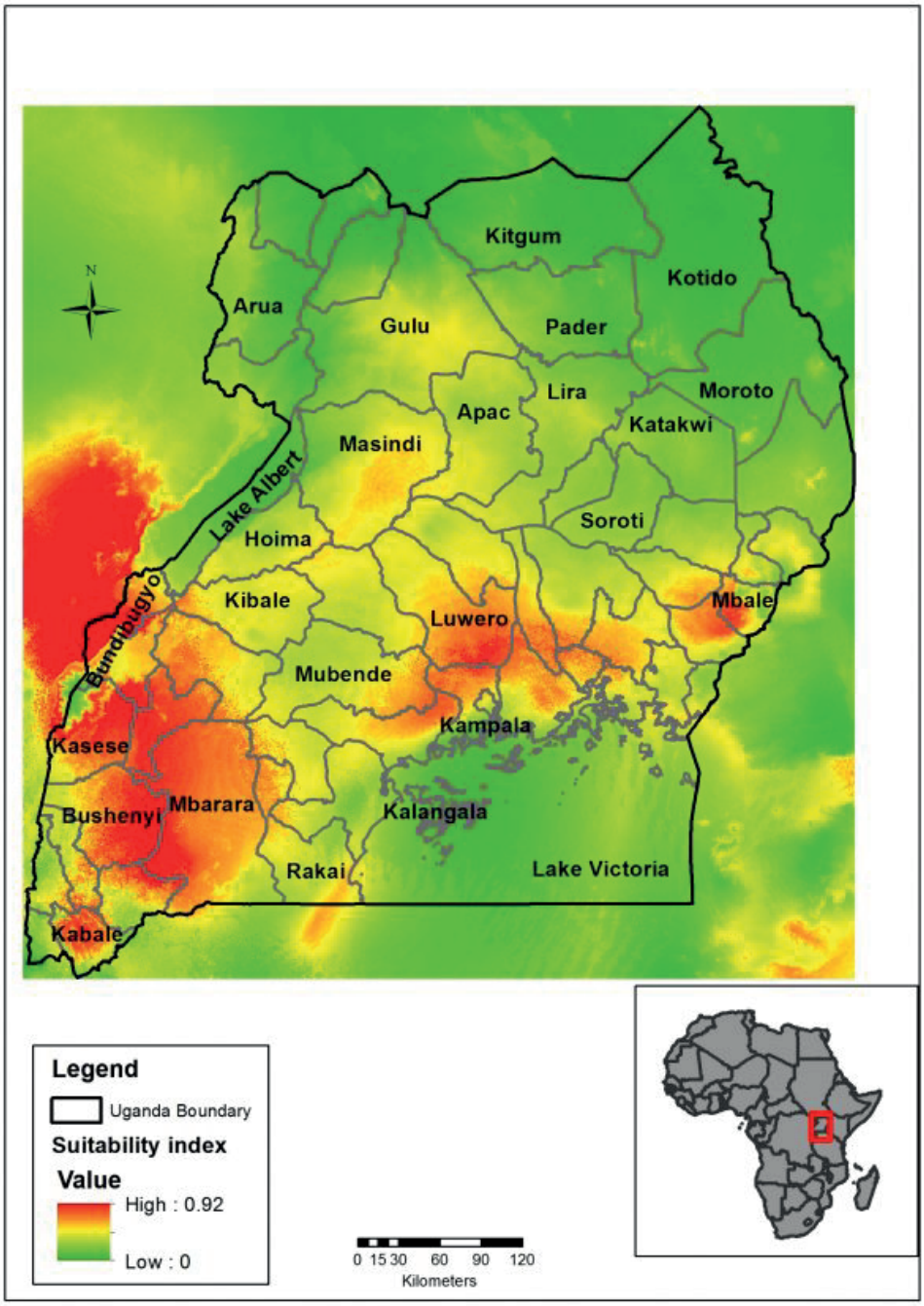

Fig. 3: Map showing areas of the relative probability of the presence of filovirus (Ebola and Marburg virus) outbreak in Uganda.

(AUC=0.9)

Variable Contribution to the prediction models

Figure 4, shows the response curve of the most important variable for each of the models (The response curves of all the predictor variables in all the four models are in Supporting Information S4 File; see Appendix). The response curves show the mean response of the 100 replicate MaxEnt runs (red) and the mean +/- one standard deviation. Figure 4A suggests that probability of bats occurrence are optimal at 30 – 40 degree Celcius during the driest quarter(Bio17). MVD occurs in areas where temperature variability (Bio4) is minimal (Figure 4C) whereas EVD (Figure 4B) and both the filovirus (Figure 4D) occurs in areas with minimal rainfall variability (Bio15).Bio4 and Bio15 show how temperature and rainfall vary over a given year based on standard deviation. The response curves, show that MVD occurs in areas with low variability of temperature and EVD / Filoviruses occur in areas with low variability of rainfall. Bio4 contributes 68% to the relative probability of occurrence of MVD, which indicates that MVD is limited when there is high variability in temperature across the year. Rainfall variables contributed about 75% to the to the relative probability of occurrence of EVD. The results indicate that EVD is limited by the amount of rainfall received in an area. Higher rainfall increases the relative probability of occurrence of EVD.

Environmental variable	Contribution bat model (%)	Environmental variable	Contribution to Ebola model (%)
Rainfall driest quarter	24.7	Rainfall seasonality	33.2
Mean annual rainfall	17.2	Mean annual rainfall	25.7
Mean diurnal range in temp	14.9	Rainfall driest quarter	20.8
Isothermality	11.5	Mean diurnal range in temp	9.9
Rainfall seasonality	10.8	Temperature seasonality	5
Temperature seasonality	10.5	Isothermality	3.5
Mean annual temperature	10.4	Mean annual temperature	2.1
Environmental variable	**Contribution to Marburg model (%)**	**Environmental Variable**	**Contribution to filovirus model (%)**
Temperature seasonality	68.2	Rainfall seasonality	29.6
Rainfall seasonality	25.3	Rainfall driest quarter	23.2
Rainfall driest quarter	3.7	Temperature seasonality	15.7
Mean diurnal range in temp	1.2	Mean annual rainfall	14.9
Mean annual temperature	0.9	Mean diurnal range in temp	8
Mean annual rainfall	0.6	Isothermality	5.9
Isothermality	0.1	Mean annual temperature	2.7

Table 2: Environmental variable contribution in the MaxEnt prediction models

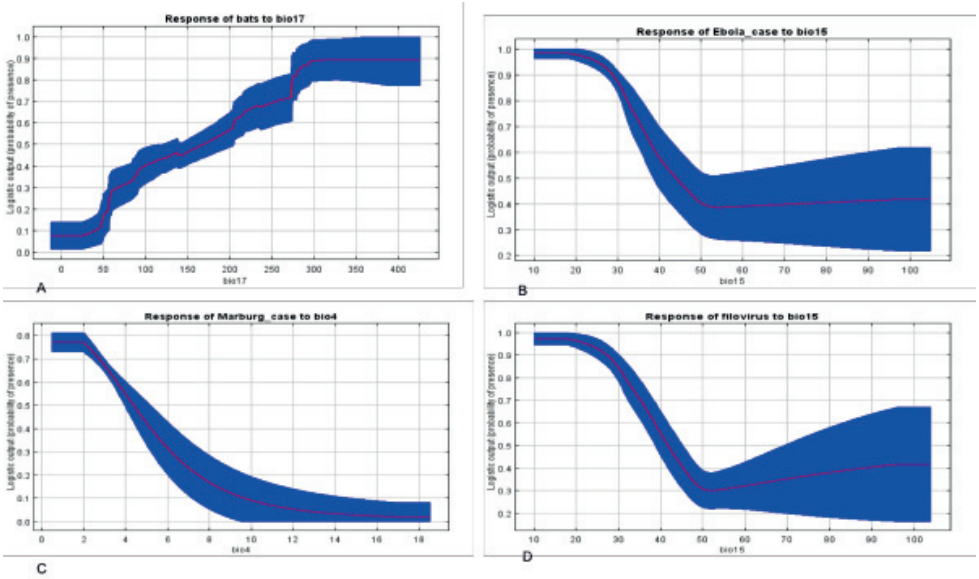

Fig. 4: Response curves of environmental variables that contribute highest to each of the prediction models.

A: Rainfall driest quarter(BIO17) vs Relative probability of bat presence. B: Rainfall seasonality(BIO15) vs. Relative probability of presence of Ebola virus outbreak; C: Temperature seasonality(BIO4) vs. Relative probability of presence of Marburg virus outbreak; D: Rainfall seasonality(BIO15) vs Relative probability of presence of Ebola or Marburg virus disease outbreak

Discussion

We used seven environmental variables in this model prediction. This was after assessing for collinearity in the model and removing all the collinear variables. Variable contribution assessment as shown in Table 2 showed that rainfall variables were the most important predictors. The importance of rainfall or precipitation and moderate to high temperature was highlighted by Peterson *et al* (2004) when they modeled filovirus distribution in Africa using GARP model[29,31]. Rainfall is important for the obvious reason that it provides water which is very important for bats survival[52,53]. Rainfall also provides for the development of fruiting trees that provide roosting areas for bats as well as food for fruit bats. Uganda is endowed with many water bodies and several rainforests, and hence bat distribution tends to be all over the country as seen in Figure 2A. Bats are hypothesized to be reservoirs for filoviruses; their distribution tends to correlate with that of filovirus predicted niches (Figure 3). Although we have some progress with Marburg virus in trying to describe bats as a source of infection for humans[7,8,10,54], more research needs to be done especially on the reservoir for Ebola virus as these models can only give a clue as to the possible surveillance sites and possible areas to focus the research and to identify other potential reservoirs for filovirus. Temperature and rainfall seasonality were the most important environmental variables contributing to spatial prediction model for the Ebola and Marburg viruses. Seasonality has been found to be key in outbreaks of filoviruses, especially MVD as was reported in an ecological study by Amman et al. 2012[8]. In this study, outbreaks of MVD are associated with the birthing seasons of adult juvenile bats when the virus circulation was high. This is further validated by a high percentage contribution (68.2%) of temperature seasonality into the MVD outbreak prediction model (Table 2). The relative probability of the presence of a Marburg outbreak is higher (80%) and at very low-temperature seasonality, which is a standard deviation (SD) over monthly values (Figure 4C). Therefore, areas with fewer variations in monthly temperature and rainfall are more likely to experience MVD and EVD outbreaks and this has been predicted by the models in Figures 2 & 3. The areas shown on the risk maps with a high relative probability of the presence of an outbreak are mainly in the South, the West and Central Uganda that have minimal temperature and rainfall variations compared to North Eastern Uganda that is not predicted for filovirus outbreaks except for bat presence. Bat presence model is mainly influenced by the variable rainfall driest quarter (24.7%) and mean annual rainfall (17.2%) (Table 2). As these variables increase, the relative probability of the presence of bats tends to increase. Areas of high rainfall are more likely to be forested or with many fruiting trees that provide a suitable habitat for bats, and this is true for three-quarters (75%) of Uganda.

Whereas Pigott *et al* (2015) used environmental covariates with a spatial resolution of 5km in their models[55,56] , we used Africlim data with 1km spatial resolution. High-resolution data increases the accuracy of the models, and this was observed in our study by a high AUC greater than 0.8 recorded in all models.

The predictions show that a big part of Uganda, a country of 34 million people is at risk of a filovirus outbreak. This is more so in the Lake Victoria basin districts and in the Albertine Rift region districts and the areas that occur in between (Figure 2 & 3). The Albertine Rift region provides a variety of habitats characteristic of the East African savannahs and the West African rain forests that are suitable for reservoirs of filoviruses. According to Uganda National Meteorological department, these are the areas that receive near or above normal seasonal rainfall, and seasonal temperature variations are minimal[57]. Moreover, we see from variable contribution (Table 2), response curves (Figure 4) and Jackknife test (Supporting Information S3 File; see Appendix) that rainfall and temperature seasonality were the most important variables in predicting outbreaks. The lower the variability in rainfall and temperature, the higher the relative probability of presence and vice versa and an increase in mean rainfall variables increases relative probability of having a filovirus outbreak (Figure 4). Indeed, six filovirus outbreaks have happened in this region, one caused by Bundibugyo ebolavirus in Bundibugyo district in the plains of Rwenzori mountains[2], Sudan Ebolavirus in Kibale district[5] and four outbreaks of Marburg virus all linked to Python cave and Kitaka gold mines in Kamwenge, Ibanda, and Rubirizi districts[6,9,58,59]. This remains a high-risk area with cross-border movement between Uganda and DRC where another EVD outbreak happened in 2012 in the neighboring Isiro region[60] The Albertine Rift of East Africa needs to remain under heightened surveillance especially now that oil exploration will be taking place bringing an invasion of virgin lands by humans and interaction of wildlife and humans. Important to note also in this region has six national parks of Uganda (Queen Elizabeth National Park, Murchison Falls National Park, Kibale

Forest National Park, Semiliki National Park, Bwindi Impenetrable National Park and Mgahinga National Park) on Uganda side and several other national parks on the DRC and Rwanda side as well as several forest reserves all of which harbor various species of bats and other possible reservoirs of filoviruses. All outbreaks of Marburg virus disease in Uganda have been investigated, and all originate from the old gold mines found in Ibanda and Kamwenge district[6,9] in the Western Rift Valley which validates MVD distribution model in Figure 2C as it shows these as high-risk areas for filovirus outbreaks. A similar finding was obtained by Peterson and Samy 2016 in a recent model using MaxEnt as they predicted Sudan Ebola virus to occur in North Western Uganda between Lake Albert and Lake Vitoria[61]. We also see areas that have not had EVD outbreaks before such as West Nile region being predicted potential areas for EVD outbreak. These include areas along River Nile and areas bordering South Sudan and DRC (Figure 2B). From Table 2, we see that rainfall variable contribute a higher percentage of the relative probability of presence for filovirus habitants. These areas receive average annual rainfall between 100-120mm and are endowed with high vegetation cover and water bodies all of which make the region conducive for reservoirs of filoviruses

Another area of high concern predicted by this model is Lake Victoria basin and districts in Nile River basin in Central districts of Uganda. Uganda has reported three outbreaks of filoviruses previously detected in these regions in the districts of Luweero[4,5] and Mpigi[11,62]. This also can be attributed to the variety of habitats provided by water bodies, forests, swamps and high presence of fruit bats and other wildlife in this region. For example, the Kasokero cave that is the habitat of many Egyptian fruit bats that are known to harbor Marburg virus is found just on the banks of Lake Victoria in Masaka district, and several pathogens have been isolated from this cave [63]. This is at the same time a highly-populated region with Uganda's capital in the middle and needs to be heightened surveillance. We also predicted other regions that have not heard outbreaks of filoviruses in the past such as the Eastern region of Mbale, Busia and Tororo districts near the Mt. Elgon regions bordering with Kenya. This also still attributed to by the presence of suitable conditions for survival of putative reservoirs of Ebola and Marburg viruses. An outbreak happened in neighboring Kenya in Kitum cave[64,65]. These newly detected hotspots need to be kept under surveillance for early outbreak detection and response.

Limitations

We build on filovirus risk mapping efforts by Pigott et al[32,33,56] and Peterson et al[29,31,61] all of which have been done at the continental level of Africa. Their work was more ecologically oriented and more focused on identifying the ecological niche of species, they lacked country specific details that we bring in this publication with a bias in public health surveillance and outbreak detection rather that ecological niche identification. For public health surveillance of a country like Uganda, all filovirus species (Marburg virus, 5 Ebola virus species) are of public health importance. This makes our models more sensitive as opposed to specific risk map and hence more useful tools to the surveillance activities. There is already enough evidence of filovirus outbreaks in Uganda, especially areas predicted by our models. Focused surveillance needs to be done in these areas and bring additional surveillance in other new predicted areas where we have not heard outbreaks before. So we think modeling the map at a genus level (filovirus) level as opposed to species level is more informative for surveillance but may not be the best for ecological studies for which is not the purpose of this study. We know that disease outbreak is a combination of very many factors, not only suitable environmental covariates. However, we were not able to include as many factors as possible in this model because of lack of or poor quality data for Uganda specifically. We did not use bats as a predictor in our model because of their widespread distribution all over Uganda, otherwise doing this would lead to misleading interpretation and bias of potential outbreak hotspots as being the whole country. Another point would have been good to include in the prediction model are socio-economic factors since they play a big role in the outbreak of filoviruses.

Conclusion

Ecological niche modeling techniques have been widely used in predicting where disease outbreaks are likely to occur, more specifically where species have suitable living conditions depending on their environmental factors. The MaxEnt modeling algorithm uses presence only occurrence data and has been useful to estimate species' niche in environmental space where absence records for a species are not available as it is the case

with filoviruses. Given the public and global importance of filoviruses, developing models that predict where they are likely to occur is very important, and efforts in this direction have been done focusing on the African continent. In this paper, however, we focus on Uganda as one of the affected countries; and develop a country-specific prediction map. We show which places in Uganda that are hot spots for filovirus disease outbreaks and hence a focus on surveillance for early detection. Until now, no verified true reservoir for Ebola virus has been identified, and studies in this direction are still ongoing. In the absence of a known reservoir, these risk maps will help in early focused surveillance and early detection to avoid a global catastrophe like it happened in West Africa in 2014. Minimal seasonal variations in temperature and rainfall were important predictors of a filovirus outbreak. We believe these risk maps will be important in targeted surveillance, research and epidemic preparedness for Uganda. The results from this study also confirm previous findings that suggest that Filoviruses are mainly limited by the amount of rainfall received in an area.

Appendix

Supporting Information

S1 File: https://doi.org/10.6084/m9.figshare.5306875

S2 File: https://doi.org/10.6084/m9.figshare.5306908

S3 File: https://doi.org/10.6084/m9.figshare.5306914

S4 File: https://doi.org/10.6084/m9.figshare.5306932

Corresponding Author

Dr. Luke Nyakarahuka: n3luke@covab.mak.ac.ug; nyakarahuka@gmail.com

Competing Interests

The authors have declared that no competing interests exist.

Data Availability

All data is available in the paper and supporting files which can be found on figshare as follows: S1 File: Occurrence dataset used (Filovirus and Bats Occurrence coordinates) (10.6084/m9.figshare.5306875 <https://doi.org/10.6084/m9.figshare.5306875>)**;** S2 File: Results of the quantitative comparisons of environmental variables to test for multicollinearity (10.6084/m9.figshare.5306908 <https://doi.org/10.6084/m9.figshare.5306908>); S3 File: A jackknife test result to evaluate individual covariate importance in the model developments (10.6084/m9.figshare.5306914 <https://doi.org/10.6084/m9.figshare.5306914>); S4 File: The response curves of all the predictor variables in all the four models (10.6084/m9.figshare.5306932 <https://doi.org/10.6084/m9.figshare.5306932>).

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PAPER III

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Knowledge and attitude towards Ebola and Marburg virus diseases in Uganda using quantitative and participatory epidemiology techniques

Luke Nyakarahuka[1,2,3]*, Eystein Skjerve[1], Daisy Nabadda[2], Doreen Chilolo Sitali[4], Chisoni Mumba[4], Frank N. Mwiine[5], Julius J. Lutwama[3], Stephen Balinandi[6], Trevor Shoemaker[6], Clovice Kankya[2]

1 Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Oslo, Norway, **2** College of Veterinary Medicine, Animal Resources and Biosecurity, Department of Biosecurity, Ecosystems and Veterinary Public Health, Makerere University, Kampala, Uganda, **3** Department of Arbovirology, Emerging and Re-emerging diseases, Uganda Virus Research Institute, Entebbe, Uganda, **4** University of Zambia, School of Veterinary Medicine, Department of Disease Control, Lusaka, Zambia, **5** College of Veterinary Medicine, Animal Resources and Biosecurity, Department of Biomolecular Resources and Biolab Sciences, Makerere University, Kampala, Uganda, **6** Viral Special Pathogens Branch, United States Centers for Disease Control and Prevention(CDC), Viral Hemorrhagic Fever Laboratory, Entebbe, Uganda

* nyakarahuka@gmail.com

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Abstract

Background

Uganda has reported five (5) Ebola virus disease outbreaks and three (3) Marburg virus disease outbreaks from 2000 to 2016. Peoples' knowledge and attitude towards Ebola and Marburg virus disease impact on control and prevention measures especially during outbreaks. We describe knowledge and attitude towards Ebola and Marburg virus outbreaks in two affected communities in Uganda to inform future outbreak responses and help in the design of health education and communication messages.

Methods

The study was a community survey done in Luweero, Ibanda and Kamwenge districts that have experienced outbreaks of Ebola and Marburg virus diseases. Quantitative data were collected using a structured questionnaire and triangulated with qualitative participatory epidemiology techniques to gain a communities' knowledge and attitude towards Ebola and Marburg virus disease.

Results

Out of 740 respondents, 48.5% (359/740) were categorized as being knowledgeable about Ebola and Marburg virus diseases, whereas 60.5% (448/740) were having a positive attitude towards control and prevention of Ebola and Marburg virus diseases. The mean knowledge and attitude percentage scores were 54.3 (SD = 23.5, 95%CI = 52.6–56.0) and 69.9

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(SD = 16.9, 95%CI = 68.9–71.1) respectively. People educated beyond primary school were more likely to be knowledgeable about Ebola and Marburg virus disease than those who did not attain any formal education (OR = 3.6, 95%CI = 2.1–6.1). Qualitative data revealed that communities describe Ebola and Marburg virus diseases as very severe diseases with no cure and they believe the diseases spread so fast. Respondents reported fear and stigma suffered by survivors, their families and the broader community due to these diseases.

Conclusion

Communities in Uganda affected by filovirus outbreaks have moderate knowledge about these diseases and have a positive attitude towards practices to prevent and control Ebola and Marburg viral diseases. The public health sector should enhance this community knowledge gap to empower them more by supplying educational materials for epidemic preparedness in future using appropriate communication channels as proposed by the communities.

Author summary

Ebola Virus Disease (EVD) and Marburg Virus Disease (MVD) are caused by a family of viruses known as Filoviruses. When they occur, they cause high lethality among infected people, which causes panic to the population, as well as interfering with the health care delivery system, especially in developing countries. Usually, at the beginning of these infections, the affected communities tend to think that witchcraft or some bad luck has befallen their community. Because of limited knowledge about these diseases, it becomes hard for local authorities to institute control and prevention measures. We administered a questionnaire to individual participants and held focus group discussions to help us tease out the communities' understanding of these diseases. We found that people have moderate knowledge about EVD and MVD, and a positive attitude towards the prevention and control measures instituted by health authorities in Uganda. However, they still reported stigma subjected to the survivors of these diseases and affected families at large. EVD still causes much fear which drives some of the irrational actions by communities during outbreaks. Communities highlighted early sensitization as a means of controlling outbreaks. We recommend the findings in this paper to public health authorities in epidemic-prone countries like Uganda to aid in control and epidemic preparedness of filovirus outbreaks.

Introduction

Ebola and Marburg virus diseases are viral hemorrhagic fevers (VHFs) known to cause high morbidity and mortality and pose a serious threat to human and animal populations in endemic countries. These classical VHFs are caused by filoviruses that belong to the family *Filoviridae*. A total of 28,646 people were reported to be infected with Ebola virus in the recent outbreak in West Africa in 2014, out of which 11,323 died [1]. Apart from causing morbidity and mortality, outbreaks of VHFs cause panic among the public, interfere with global travel and have a devastating socio-economic impact [2, 3]. Uganda has reported five (5) Ebola virus

disease (EVD) outbreaks and three (3) Marburg virus disease (MVD) outbreaks since 2000. The first EVD outbreak in the year 2000 remains the largest EVD outbreak ever recorded in Uganda, during which 425 cases and 224 deaths (CFR 53%) were reported [4]. Since then, four (4) additional EVD outbreaks have occurred; one in Bundibugyo district in 2007 caused by *Bundibugyo ebolavirus* (116 cases, 39 deaths) [5]. Other outbreaks happened in Luweero district in 2011 (one case, one death) [6], in Kibaale district in 2012 (11 confirmed cases, four deaths) and Luweero district again in 2012 (6 cases and three deaths) [7].

Three (3) MVD outbreaks have been reported in Uganda. The first recorded outbreak was in 2007, where three (3) cases and one (1) death were reported in a community associated with mining activities in the districts of Kamwenge and Ibanda, Western Uganda [8]. In 2012, MVD was responsible for 26 cases with 15 deaths affecting multiple districts [9]. In 2014, Uganda reported only one case diagnosed with Marburg virus in Kampala (Uganda's capital city) [10]. These outbreaks are believed to occur because of close interaction of people and animals such as non-human primates, bats, and livestock. Previous studies in Uganda have demonstrated bats of species *Rousettus aegyptiacus* to be the known reservoir for Marburg virus [11–13]. In Uganda, this bat species has been found in the Kitaka mine in Ibanda district as well as in Maramagambo "python cave" in the neighboring Rubirizi district, as well as other sites in the surrounding areas. Two tourists visiting python caves were infected with Marburg virus in 2008 with one fatality [14–16].

These outbreaks cause loss of human life, associated morbidities and induce stress on the socio-cultural and health care systems as efforts to respond to these outbreaks require many resources such as funds, laboratory testing, and personnel. Usually, when these outbreaks occur, health care workers run away from health facilities leaving patients with no health care and support due to lack of protective equipment, fear of contracting the disease and stigmatization from their families [17].

Research done in West Africa by Iliyasu *et al*. [18] showed suboptimal knowledge, attitudes and practices towards EVD, and associated myths and misconceptions which negatively impacted the response mechanisms. The stigma associated with communicable diseases like EVD interfere with control and prevention of these diseases as observed by Davtyan *et al*. [19]. Many people are reluctant to associate themselves with EVD survivors. This was the situation in the 2014 West African EVD outbreak [20, 21], also reported by deVries *et al*. 2016 in Luweero district of Uganda [22]. However, as an outbreak progresses, people tend to modify their behavior. For example, an outbreak of EVD that happened in Uganda in Masindi District 2000, the case fatality rate was high at the beginning of the outbreak (76%) but decreased to 20% at the end of the epidemic as people started modifying their behavior towards the epidemic. [23].

In Uganda, EVD survivors reported fear, ostracism, and stigmatization from their community [24]. There is always an over-reaction in communities characterized by anger, fear and the communities tend to run away from hospitals searching for spiritual healing as they associate EVD or MVD with witchcraft also locally known as "*amayembe*"[22]. These actions are counterproductive towards efforts to control the spread of VHFs.

For a better response to future EVD and MVD outbreaks in Uganda, there is a need to better understand communities' knowledge and attitudes towards these VHFs. Therefore, our main objective was to describe knowledge and attitudes in two communities affected by outbreaks of Ebola and Marburg viral diseases in Uganda. This information may be critical in designing health education, information, and communication materials in future outbreaks, leading to better control of future epidemics.

Methods and materials

Study site, study population, and sampling strategy

The study was undertaken in two different locations in Uganda in the months of January and February 2015, as part of a larger study intended to assess the seroprevalence of MVD and EVD in high-risk areas in Uganda (Fig 1). First, we focused on communities in Western Uganda in the districts of Kamwenge and Ibanda that were affected by MVD outbreaks twice, the first one in 2007 and another one in 2012. The second study site was in Luweero district, Central Uganda that has been affected by EVD outbreaks twice, one in 2011 and another in 2012 [6, 7]. The main economic activity in the two sites is agriculture, mainly crop farming and livestock keeping.

We estimated the necessary sample size using StatCalc, an application in the EpiInfo software, which gave us 768 study participants, 384 in each study area, based on an expected proportion of the population that have knowledge about MVD and EVD at 50% and the desired precision of 5%. However, only 740 completed the questionnaires representing a response rate of 96.4%. We studied the population in Ibanda, Kamwenge and Luweero districts, purposively sampling villages that were affected by Ebola and Marburg virus diseases

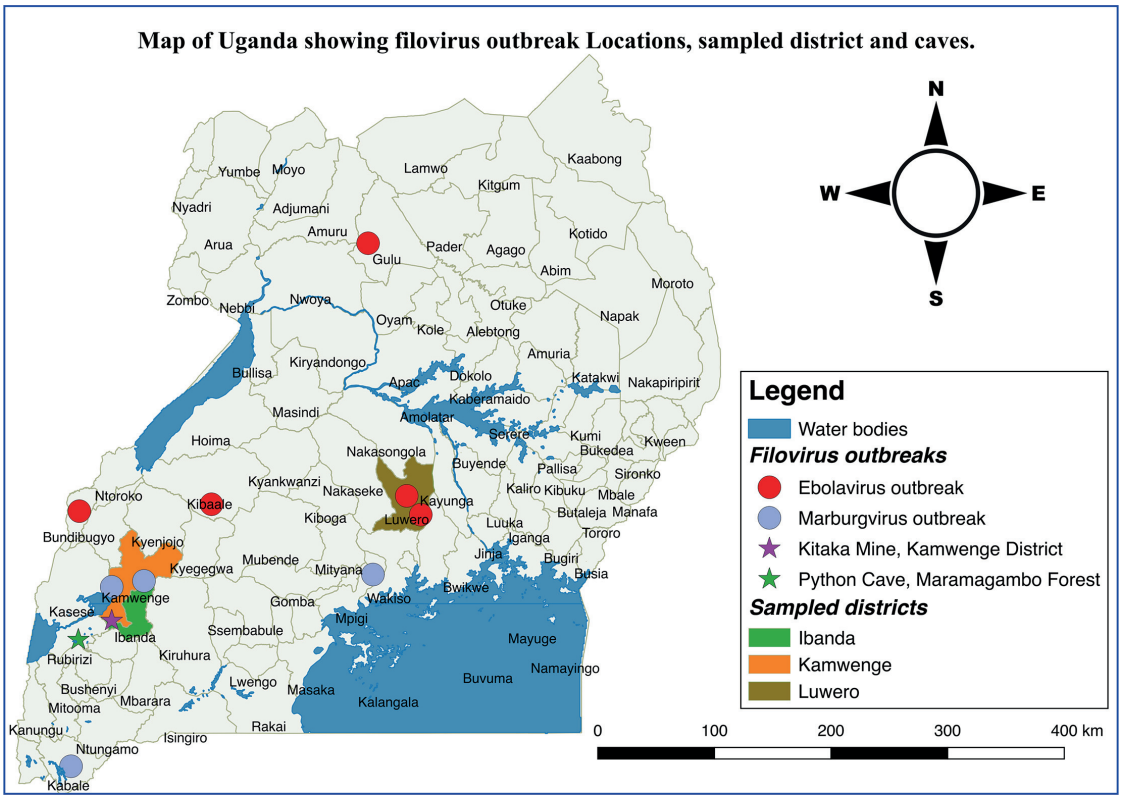

Fig 1. Map of Uganda showing Ebola and Marburg disease outbreaks and study districts (map developed in QGIS desktop software, the base layers from Uganda bureau of statistics-http://www.ubos.org/statistical-activities/gis/).

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outbreaks. A multisector team of members from the Uganda Virus Research Institute (UVRI), Ministry of Health Uganda, Makerere University Kampala Uganda and US Centers for Disease Control and Prevention Uganda working with district health teams visited affected villages to recruit study participants. Through working with local community leaders and health workers, a snowball approach was used to recruit participants. Participants for the questionnaire were chosen using convenient sampling, and both communities were asked the same questions.

Quantitative data collection

Research assistants were trained to use a structured questionnaire to collect data (S1 Questionnaire). Participants were asked to give a written consent after the objectives of the study were explained to them before the questionnaire could be administered. The questionnaire was pretested in Wakiso district that was not part of the survey to ensure that validity and clarity of the questions, and minor editing was done to get a final questionnaire. The questionnaire consisted of three sections, socio-demographic characteristics, practices that predispose people to EVD and MVD, knowledge and attitude questions. Closed-ended questions were used to assess peoples' knowledge and attitudes on transmission and risk factors, prevention and control, causation, signs and symptoms and treatment of MVD and EVD. Questionnaires were administered in the local language to one person per household that lived in sub-counties that had reports of EVD and MVD outbreaks but not to survivors or their family members as these were targeted for qualitative data collection.

Attitude and knowledge scoring

Knowledge and attitude questions that were answered correctly were scored one (1) while those that were answered wrongly were scored zero (0). All questions were given equal weight, and missing responses were not scored, whereas "do not know" responses were scored zero (0). The knowledge and attitude score for each study participant were used to compute the percentage scores out of a total score of 34 and 20 respectively. The validity of the knowledge and attitude questions was confirmed by an adequate Cronbach's alpha internal consistency measured at 0.90.

Quantitative statistical analysis

Data were entered into EpiInfo software, assessed for normality and univariate analysis was done and later exported to Stata (Stata/ SE for Windows, StataCorp, College Station, TX) for further analysis. Results are presented in tables and narratives. A cut-off point was set based on percentage knowledge and attitude distribution, and median scores as was described in other studies [18, 25]. For knowledge score, the median percentage score was 56%; with a bimodal curve distribution of the scores, hence those below a 56% score were categorized as having poor knowledge and those with 56% and above score as having good knowledge (Fig 2A). Further, attitudes were classified as being negative if the percentage score was below the median score of 70% and positive if the median score was 70% and above (Fig 2B). The relationship between good or poor knowledge and attitude was explored using a univariable logistic regression. Predictors of good versus poor knowledge with a p-value of 0.2 and below were included in a multivariable logistic regression model to determine the predictors of good knowledge towards EVD and MVD. The model was constructed using a backward selection procedure using the likelihood ratio test (LRT) with a $p = 0.05$ for keeping a variable in the model. Model evaluation was done using the Hosmer-Lemeshow test of goodness of fit and the area under the receiver operating curve (ROC).

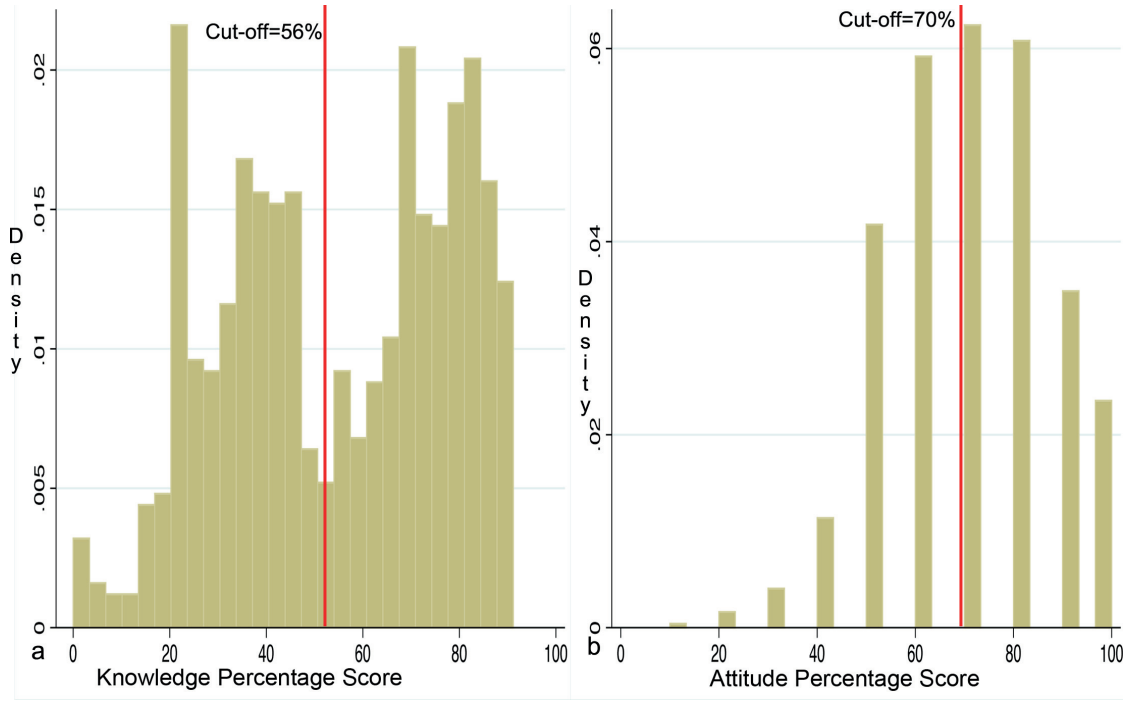

Fig 2. a: Distribution of percentage knowledge scores; the red line shows cut-off set at a median score of 56%. b: Distribution of percentage attitude scores; the red line shows cut-off set at a median score of 70%.

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Participatory epidemiology data collection techniques

Qualitative participatory appraisal techniques, also known as Participatory Epidemiology (PE) were used to triangulate the findings of quantitative data collection. Five (5) focus group discussions (FGDs) involving 50 participants were conducted. FGDs were held within rural communities that were affected by outbreaks, drawn mainly from survivors of EVD and MVD and their family members, community and opinion leaders, as well as other members of the community who were 18 years and above. The discussions involved both male and female respondents since gender disaggregation was not the focus of this study.

An introduction explaining the purpose of the exercise was carried out with the informants before conducting the interview. Semi-structured interview guides were translated into the community's local language (Luganda and Runyankore) by trained research assistants and were used to gain an understanding of the local perception of Ebola and Marburg virus diseases (S1 FGD guide). To get a clear knowledge of the community's knowledge and attitude towards EVD and MVD, we subjected the information generated from FGD guide to three PE tools, which included simple ranking, proportional piling, and pairwise ranking. Simple ranking techniques helped us to understand what the community considered as the most important depending on the topic being discussed. For example, the FGD participants were asked to list what they believed to be the clinical symptoms of EVD and MVD and later requested to rank them from the most important to the least important according to their opinion (S1 Fig).

Proportional piling was used to study what the community thinks are modes of transmission of filoviruses. Here, participants were given 100 grains of beans and were required to distribute them according to the importance of the factor being discussed. Informants did not count the beans; rather they simply piled the beans judging by the importance of the mode of transmission in spreading filoviruses (S2 Fig). The pairwise ranking technique was used to understand the communities' ideas on the source, cause or the triggers of EVD and MVD outbreaks. Pairwise ranking technique compared each proposed source or cause of the outbreaks with each other systematically and then ranking was done to see what the community considers as the most important cause of outbreaks in their communities (S3 Fig). Agreement within FGDs participants was reached by consensus.

Discussions from FGDs were audiotaped with permission from informants and transcribed verbatim. Data generated through FGDs were analyzed using conventional content analysis as reported by Hsieh and Shannon [26] where qualitative data was merged into codes, categories and themes. Text data were read several times to get a deeper understanding of the emerging codes and categories. Categories were later grouped into topics such as participants understanding of Ebola and Marburg virus diseases, modes of transmission, clinical symptoms, the impact of outbreaks, communication, prevention, and control.

Ethics statement

Approval to conduct this study was obtained from Uganda Virus Research Institute Research and Ethics Committee and Uganda National Council of Science and Technology (UNCST approval NO: HS 1538). Participants gave signed written consent to participate in this study. For participants under the age of 18 years, informed consent was provided by their parents or their guardians on their behalf.

Results

Socio-demographic characteristics of questionnaire survey participants

Of the 740 participants who completed the questionnaire, 60% were from Western Uganda in communities affected by MVD in Ibanda and Kamwenge districts and 40% were from Central Uganda in EVD affected communities of Luweero district. Overall, 54.2% were males, 16.8% had never attended any formal education, and the majority (62.7%) occupation was farming. The median age was 33 years (range 3–82 years), and 85.2% were above 20 years. These statistics are close to those of Uganda population census 2014 from these districts.

Knowledge on Ebola and Marburg viral diseases and their modes of transmission

Table 1 highlights some of the responses from participants on questions assessing knowledge about Ebola and Marburg virus diseases and their modes of transmission. Almost all (96.2%) had heard about EVD and MVD, 43.5% reported to know how to identify a suspect case of EVD and MVD, the most known clinical symptom for EVD and MVD was bleeding at 54.3%, and 28.2% reported to know a survivor of EVD and MVD.

On the mode of transmission, 51% knew how EVD and MVD are transmitted. A total of 54.2% knew that EVD or MVD could be transmitted through body contact with an infected person, while 11.3% thought that EVD/MVD could be transmitted through biting mosquitoes, 16.7% thought that EVD/MVD are airborne, 50.9% mentioned that it could be transmitted through semen or sexual contact and 53.3% knew that EVD/MVD could be transmitted through breast milk of an infected person.

Table 1. Knowledge on Ebola and Marburg viral diseases and their modes of transmission.

Variable	n/N	Percent (%)	95% Confidence Limits
Have heard about Ebola & Marburg virus diseases			
Yes	712/740	96.2%	94.5%-97.4%
No	28/740	3.8%	2.6%-5.5%
Source of information about Ebola & Marburg virus diseases			
Health worker	113/724	15.6%	13.1%-18.4%
Radio	614/726	84.6%	81.6%-87.1%
Community leaders	146/724	20.2%	17.3%-23.3%
Other sources of communication	64/720	8.9%	6.9%-11.3%
Know symptoms of Ebola and Marburg virus disease suspects			
No	392/740	53.0%	49.3%-56.6%
Not Sure	26/740	3.5%	2.3%-5.2%
Yes	322/740	43.5%	39.9%-47.2%
Known symptoms of Ebola and Marburg virus disease			
Bleeding	277/510	54.3%	49.8%-58.7%
Fever	106/508	20.9%	17.4%-24.7%
Vomiting	100/509	19.7%	16.3%-23.4%
Diarrhea	87/506	17.2%	14.1%-20.8%
Other signs	52/501	10.4%	7.9%-13.5%
Know whom to contact for suspect case of Ebola and Marburg virus diseases			
Yes	50/740	6.8%	5.1%-8.9%
No	690/740	93.2%	91.13%- 94.9%
Know a survivor of Ebola or Marburg virus diseases			
Yes	209/740	28.2%	25.1%- 31.7%
No	531/740	71.8%	68.3%-75.0%
Know how Ebola or Marburg virus diseases are transmitted			
No	327/739	44.3%	40.6%-47.9%
Not sure	35/739	4.7%	3.4%-06.6%
Yes	377/739	51.0%	47.4%-54.7%
Known modes of transmission of Ebola and Marburg virus disease			
Body contact with Ebola infected person	289/533	54.2%	49.9%-58.5%
Through air	90/533	16.9%	13.7%-20.4%
Through needle pricks	71/532	13.4%	10.6%-16.6%
Contact with animals	162/534	30.3%	26.5%- 34.5%
From a person who died of EVD or MVD	122/532	22.9%	19.5%-26.8%
Contact with body fluids of sick person	134/533	25.1%	21.6%-29.1%
Biting mosquitoes	60/530	11.3%	8.8%-14.4%
Other means of transmission	40/524	7.6%	5.6%-10.3%
Think one can get infection from asymptomatic Ebola or Marburg virus disease suspects			
No	167/530	31.5%	27.6%-35.7%
Not sure	19/530	3.6%	2.2%-5.6%
Yes	344/530	64.9%	60.7%-68.9%
Think one can acquire Ebola or Marburg virus disease from contact with bush meat			
No	203/543	37.4%	33.3%-41.6%
Not sure	42/543	7.7%	5.7%-10.4%
Yes	298/543	54.9%	50.6%-59.1%
Think one can get Ebola or Marburg virus disease from eating fruits eaten on by bats			
No	201/541	37.2%	33.1%-41.4%

(*Continued*)

Table 1. (*Continued*)

Variable	n/N	Percent (%)	95% Confidence Limits
Not sure	40/541	7.4%	5.4%-10.0%
Yes	300/541	55.5%	51.2%-59.7%
Think one gets infected from sexual fluids of a person who recovered from infection			
No	202/540	37.4%	33.3%-41.2%
Not sure	63/540	11.7%	9.1%-14.8%
Yes	275/540	50.9%	46.6%-55.3%
Think one gets infected from breast milk of an infected person or survivor			
No	207/539	38.4%	34.3%-42.7%
Not sure	45/539	8.4%	6.2%-11.1%
Yes	287/539	53.3%	48.9%-57.5%
Shaking hands/physical contact with a person infected with Ebola or Marburg viruses			
No	167/538	31.0%	27.2%-35.5%
Not sure	20/538	3.7%	2.4% -5.8%
Yes	351/538	65.2%	61.0%-69.2%

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Knowledge on control and prevention of Ebola and Marburg virus diseases

A total of 62.8% (465/740) reported to know how EVD and MVD could be controlled and prevented, 52.8% (362/686) said by avoiding sick people, 39.4% (270/686) by avoiding contact with animals and 11.1% (76/686) by vaccination. Only 4.5% (24/531) knew that EVD and MVD are caused by a virus, 58.7% thought that it is caused by wildlife such as primates and monkeys whereas only 1.1% attributed it to witchcraft as shown in Table 2.

Attitudes towards Ebola and Marburg viral disease

Regarding attitude, 87.3% (646/740) of participants believed that EVD and MVD actually exist, 52.7% (386/733) would not relate with a survivor of Ebola or Marburg virus disease. The fear of contracting the disease was the main reason for not associating with EVD, or MVD survivors representing 59.6% (334/560), 24.7% (182/736) would not welcome a survivor back into the community as shown in Table 3.

Overall knowledge and attitude towards Ebola and Marburg Virus diseases

Out of 740 respondents, 48.5% (359/740) were categorized as being knowledgeable about Ebola and Marburg virus diseases whereas 60.5% (448/740) as having a positive attitude towards control and prevention of Ebola and Marburg viral diseases. The mean knowledge and attitude percentage scores were 54.3 (95%CI = 52.6–56.0) and 69.9 (95% CI = 68.9–71.1) respectively.

Table 4 shows results from the logistic regression model for the predictors of knowledge about EVD and MVD, identified as being male, attaining secondary and post-secondary levels of education. The Hosmer-Lemeshow test for goodness of fit shows that the model fits very well the data (P-value = 0.93), and area under the ROC curve = 0.71(S4 Fig). Results of the regression model using uncategorized knowledge percentage scores were the same as the logistic regression model(S3 Table).

Table 2. Knowledge on control and prevention of Ebola and Marburg virus disease.

Variable	n/N	Percent (%)	95% Confidence Limits
Reported to know control and prevention measures			
No	218/740	29.5%	26.2%-32.9%
Not sure	57/740	7.7%	5.9%-9.9%
Yes	465/740	62.8%	59.2%-66.3%
Known control and prevention measures			
Vaccination	76/686	11.1%	8.9%-13.7%
Avoiding contact with animals	270/686	39.4%	35.7%-43.1%
Traditional medicine	22/685	3.2%	2.1%-4.9%
Avoiding sick people	362/686	52.8%	48.0%-56.6%
Other means	90/686	13.1%	10.7%-15.9%
Know the cause of Ebola and Marburg viruses			
No	323/727	44.4%	40.8%-48.1%
Not sure	52/727	7.2%	5.4%-9.3%
Yes	352/727	48.4%	44.7%-52.1%
Known causes of Ebola and Marburg viruses			
Virus	24/531	4.5%	3.0%-6.8%
Bats, monkey or other wild animals	312/532	58.7%	54.3%-62.9%
God or other higher power	9/531	1.7%	0.8%-3.3%
Witchcraft	6/531	1.1%	0.5%-2.6%
Evil-doing	4/531	0.8%	0.2%-2.1%
Curse	4/531	0.8%	0.2%-2.1%
Prevention by avoiding contact with body fluids			
No	157/596	26.3%	22.9%-30.1%
Not sure	019/596	3.2%	2.0%-5.0%
Yes	420/596	70.5%	66.6%-74.1%
Prevention by avoiding funerals			
No	180/590	30.5%	26.9%-34.4%
Not sure	33/590	5.6%	3.9%-7.9%
Yes	377/590	63.9%	59.9%-67.8%
Prevention by reporting suspects to hospital			
No	162/598	27.1%	23.6%-30.9%
Not sure	26/590	4.4%	2.9%-6.4%
Yes	410/590	68.6%	64.7%-72.3%

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Participatory epidemiology results

Peoples' Beliefs about Ebola and Marburg virus diseases. Participants were asked questions regarding knowledge and attitude towards Ebola and Marburg virus diseases, and their discussions are summarized in S1 Table. People believed that Ebola and Marburg viral diseases kill instantly, cause chaos, and are more severe than HIV. There is much fear when the word "Ebola" is mentioned as it is considered a terrible disease.

"*When I hear Ebola, I lose strength because it kills instantly*," said one of the participants in FGD 2.

"*When you get Ebola, your life ends there*," retorted another participant in FGD 1. The details of themes, categories, and quotes are presented in supporting information file(S1 Table)

Table 3. Attitudes towards Ebola and Marburg viral disease.

Variable	Frequency (n/N)	Percent (%)	95% Confidence Interval
Believe that Ebola and Marburg viral diseases really exists			
No	48/740	6.5%	4.9%-8.6%
Not sure	46/740	6.2%	4.6%-8.3%
Yes	646/740	87.3%	84.3–89.6%%
Would relate with survivor of Marburg and Ebola viral disease			
No	386/733	52.7%	49.0%-56.3%
Not sure	26/733	3.6%	2.4%-5.2%
Yes	321/733	43.8%	40.2%-47.5%
Why they would not relate with survivor of EVD/MVD			
Fear of contracting the disease	334/560	59.6%	55.4%-63.7%
Fear of stigma from community	18/554	3.3%	2.0%-5.2%
Other reasons	6/551	1.1%	0.4%-2.5%
How Ebola and Marburg viral diseases should be treated			
Traditional African medicine	3/706	0.4%	0.1%-1.3%
Spiritual healing	8/710	1.1%	0.5%-2.3%
Modern Western medicine	652/711	91.7%	89.4%-93.6%
Herbal medicine	5/708	0.7%	0.3%-1.7%
Other modes of treatment	17/704	2.4%	1.5%-3.9%
Think are at risk of infection with Ebola or Marburg virus diseases			
No	159/739	21.5%	18.6%-24.7%
Not sure	67/739	9.1%	7.1%-11.4%
Yes	513/739	69.4%	65.9%-72.7%
Would buy from a shopkeeper who is a survivor			
No	298/740	40.3%	36.7%-43.9%
Not sure	29/740	3.9%	2.7%-5.7%
Yes	413/740	55.8%	52.1%-59.4%
Would keep information secret if family member is suspected to be infected with EVD or MVD			
No	465/722	64.4%	60.8%-67.9%
Not sure	25/722	03.5%	2.3%-5.5%
Yes	232/722	32.1%	28.8%-35.7%
Would welcome back a survivor of Ebola or Marburg virus disease into the community			
No	182/736	24.7%	21.7%-28.0%
Not sure	26/736	3.5%	2.4%-5.2%
Yes	528/736	71.7%	68.3%-74.9%

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Knowledge on cause and mode of transmission of Ebola and Marburg viral diseases. Almost all the participants agreed that EVD and MVD spread very fast and are highly infectious and contagious diseases. They appreciated the need not to conduct and participate in any funeral rites whenever loved ones die. However, they found this position very hard to accept.

Communities identified non-human primates such as monkeys, chimpanzees and other wildlife such as bats as sources of Ebola and Marburg viral outbreaks as shown by pairwise ranking in S2 Table. However, some people believe that EVD and MVD are transmitted through the air (airborne), poor hygiene, and some think foreign doctors can spread it by malice.

Table 5 shows the results of simple ranking procedure of what the communities believe are the clinical signs of Ebola and Marburg virus disease. Almost 50% think EVD is transmitted by contact with infected person. Top-ranked signs include bleeding from body orifices and other

Table 4. Logistic regression model for predictors of knowledge about EVD and MVD in Uganda (Hosmer-Lemeshow χ^2 = 1.31; p-value = 0.93, area under the ROC curve = 0.7).

Variable	Poor Knowledge (%)	Good Knowledge (%)	Total	Crude OR(95%CI)	Adjusted[a] OR (95% CI)
Gender					
Female	216(63.9%)	122(36.1%)	338(45.7%)	Ref	
Male	165(41.0%)	237(58.9%)	402(54.3%)	2.5(1.9–3.4)*	1.9(1.4–2.6)*
Education Level					
Never attained formal Education	87(70.2%)	37(29.8%)	124(16.8%)	Ref	
Primary level of education	223(54.7%)	185(45.3%)	408(55.1%)	1.9(1.3–3.0)*	1.5(0.9–2.4)
Secondary level of education	67(35.5%)	122(65.5%)	189(25.5%)	4.3(2.6–6.9)*	3.8(2.3–6.3)*
Tertiary level of education	4(21.1%)	15(78.9%)	19(2.6%)	8.8(2.7–28.4)*	8.4(2.5–27.5)*
Occupation					
Non-miners	329(57.1%)	247(42.9)	577(77.8%)	Ref	
Miners	52(31.7%)	112(68.3)	164(22.2%)	2.9(1.9–4.1)*	2.6(1.7–3.8)*

*statistically significant

[a]adjusted for all the variables in the model

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related hemorrhagic signs, diarrhea and vomiting respectively. However, survivors believed that EVD and MVD usually start like malaria with fever.

"*I have never seen such a deadly disease since my daughter started falling sick with a simple fever and we all thought it was malaria*," said one of the participants who took care of an EVD patient in FGD 3.

Knowledge on control and prevention. Community sensitization was a major proposal fronted by the community as a way of controlling Ebola and Marburg viral diseases. They emphasize repetitive sensitization for the population to be aware of the diseases. They also emphasize safe burial of their loved ones to increase compliance. Other means of prevention

Table 5. Results of simple ranking and proportional piling as listed and ranked by five FGD participants.

Simple Ranking of Clinical Signs of Ebola and Marburg viral diseases		Impact of Ebola and Marburg viral diseases		Preferred means of communication during outbreaks		Proportional piling of modes of transmission	
Clinical signs listed by Participants	**Rank score**	**Impact listed by participants**	**Rank Score**	**Means of communication listed by Participants**	**Rank Score**	**Mode of transmission listed by participants**	**Percentage (%)**
Bleeding from body orifices	1.0	Fear of Death	1.0	Community Public Radios	1	Handshaking	49%
Diarrhea	3.0	Stigma	2.5	FM Local Radio	2	Being near an Ebola patient	22%
Vomiting	3.0	Reduced income	3.3	Village cooperative societies	3	Attending funerals	14%
Body weakness	4.3	Could not participate in Funeral rites	4.0	Local leaders	4	Taking care of the sick patients	8%
Headache	5.0	Not knowing the cause	4.0	Village meetings	5	Contact with body fluids	4%
Red eyes	5.0	No partying	4.0	TV	6	Sex with Ebola patients/ Survivor	3%
Fever	5.3	Death of people	5.0	Newspapers	7		
Anorexia	6.7	Orphans	6.0	Posters	8		
Sweating	6.3			Facebook	9		
Abdominal pain	6.7						
Sudden death	7.3						

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and control suggested by the community include quarantine, treatment, recruitment of health workers who are qualified to handle EVD and MVD. They also proposed the elimination of bats and rats and other wildlife that they believed to be causes of Marburg and Ebola viral diseases.

We also explored how the outbreaks of Ebola and Marburg impact the communities (Table 5). The community is usually engulfed with fear, especially the fear of deaths and the fear of the unknown cause of the disease. Initially, because of sudden deaths of many people especially in one family, they believe it's witchcraft. When Ebola virus is confirmed in their communities, business goes down drastically thus affecting them economically as was seen in West Africa [27–29]. The community also suffers from stigma from fellow citizens, but also if they go to hospitals, they may not receive treatment from the health workers. Other effects on the community include failure to participate in funeral rites of their loved ones, no social gatherings, and challenges associated with taking care of orphans and widows and widowers.

Regarding handling survivors of Ebola and Marburg virus disease, the communities do believe the person can recover completely from these diseases unless they have a letter from a health officer or the authority saying that the person has fully recovered. They reported that they would avoid the person for some time until they are sure the person is fully recovered.

On gender perspectives, they were contrasting views on whether it is men or women that are most affected by filovirus outbreaks. Although the majority believed that women were mostly affected, other participants said men are more affected by filovirus outbreaks. To sort out this conundrum, we used proportional piling that showed men scoring 55% compared to women with 45%. It shows that both men and women are almost equally affected during filovirus outbreaks (S5 Fig).

We explored ways on how best communication should best be done during outbreaks and community members ranked the community radio as the most effective means of communication followed by FM radio stations, village cooperative organizations and the community leaders (Table 5).

Discussion

We found that EVD/MVD affected communities in Uganda are knowledgeable about EVD/MVD at 48.5% and 60% have a positive attitude towards control and prevention of these diseases. This is slightly higher than what has been found in similar studies towards Ebola virus disease especially in West Africa [18, 20, 30–38]. This is partly because Uganda has had many outbreaks of Ebola and Marburg viral diseases, which has led to continuous sensitization of communities to these diseases, hence change in attitude, and more knowledge gained. However, the proportion categorized as knowledgeable about EVD/MVD is still below average at 48%, and more sensitization is needed if future outbreaks are to be controlled in the shortest time possible. Community support and involvement are very key in control and prevention of VHFs given that this survey was done in communities that had exposure to VHF outbreaks, the knowledge levels could even be lower in other naïve communities. There is still a big proportion (above 51.5%) that is still less knowledgeable and have negative attitudes (40%) towards control and prevention, and these results should not be over-interpreted. One would have expected higher levels of knowledge given that the studied communities have experienced filovirus outbreaks twice.

Every outbreak that occurs is an opportunity to educate communities about a given disease, and Uganda has had EVD outbreaks five times [4–7] and MVD three times [8, 10, 39]. This should have provided the Ministry of Health of Uganda and other partners an opportunity to educate these communities on the modes of transmission, clinical symptoms, putative

reservoirs and control and prevention methods. Although the communities demonstrate much fear towards Ebola and Marburg viruses, this can be advantageous for control and prevention measures as communities will be motivated to action if an outbreak occurs. However, this fear becomes counterproductive as far as survivors are concerned. Disease stigma is still an issue as 53% of the respondents said they would not associate with a survivor for fear of contracting the disease. Respondents reported that they would only associate with the survivor of EVD or MVD after careful evaluation and receiving a report from a health worker or the authority concerned. This was also observed in the 2001 EVD outbreak in Northern Uganda, as communities initially had their reservations about Ebola virus disease and survivors. However, after they had been explained to fully by the health care workers, survivors were accepted and are now living peacefully in their communities [40]. It is still hard for communities to fully accept that people completely recover from Ebola virus and that they can easily mix and interact with the rest of the community as evidenced in this research and from the West Africa EVD outbreak experience [20, 21].

Most participants mentioned that filoviruses spread fast, meaning they are highly contagious as was discussed in focused group discussions. Several modes of transmission were reported by the participants, which include contact with infected patients and contact or eating non-human primates and bats. This knowledge by the community is helpful during outbreaks in instituting control and prevention measures by health authorities. In communities that do not know modes of transmission, it would be difficult to stop the spread of the epidemic as was seen in West Africa [41]. However, we still have a few people who think that EVD and MVD are airborne, caused by witchcraft or by malice by medical workers from foreign countries. This was also revealed by de Vries *et al.* (2016) in an anthropological study in one of our study areas in Luweero district[22]. Such misconceptions need to be addressed because if taken on by opinion and community leaders as it happened in 2012 Luweero EVD outbreak, they could hamper prevention and control measures.

Participants highlighted bleeding symptoms as the most common sign of Ebola and Marburg viral disease (54%), and less than 20% indicated fever, diarrhea, and vomiting as a clinical sign of filoviruses. However, bleeding is not always there in all filovirus infected cases and usually comes at the end of the clinical course of the diseases [42]. It is important that both the public and clinicians know that hemorrhagic symptoms come later when the disease has progressed, and people who show hemorrhagic symptoms rarely recover. Early symptoms of filovirus infection are like those of any other infectious disease in the tropics, and they could easily be mistaken for malaria or typhoid. Hence mechanisms for early detection should be instituted to avoid missing cases as communities and clinicians wait to see hemorrhagic signs.

Sensitization of communities about filoviruses was the most effective means of control and prevention, as suggested by participants. They believe that if they are imparted with knowledge on the modes of transmission, control and prevention measures, and spread of the epidemic could be stopped in case there is an outbreak. They seemed not to understand though, the reasons why they could not participate in the long-held culture of funeral rites when they lose their loved ones. There is an information gap between health care providers and the affected communities on how filoviruses are transmitted and the how they should be managed. Although the community proposes other control measures such as quarantine and isolation of sick people and avoiding contact with infected patients, the feeling is that if they were fully sensitized about these methods before, during and after outbreaks would significantly reduce transmission chains during epidemics. Unlike the survey that was done in West Africa where TV was the most common source of information [20], participants in this study preferred the use of community radios as the most efficient way of passing on communication to the communities (Table 5). This model involves the use of loudspeakers placed in community trading

centers where announcements can be made. Other preferred modes of communication during outbreaks included use include of FM local radio stations, use of village health teams and community leaders. These community-based strategies could prove to be efficient in communicating filovirus outbreaks instead of putting communication on TVs and other radio stations that do not broadcast in local languages and are city-based.

As seen in West Africa EVD outbreak 2014, filovirus outbreaks can be devastating since they change from localized disease outbreaks into a humanitarian crisis [43]. In this study, we see participants reporting several effects, which include the community being engulfed with fear of death or what they described as "the fear of unknown." This fear of the unknown can lead to irrational decisions which can even potentiate the spread of the disease. This fear needs to be addressed early when the outbreak is detected and has been a missing link in many outbreaks of filoviruses. Communities tend to be isolated, and their business goes down drastically as other people from the same country do not want to associate with them. Another big complaint that is social-cultural in nature was a failure by the community to bury their loved ones. This needs to be addressed during outbreaks so that communities can feel like their loved ones have been buried in a proper way. Some African cultures believe that if someone is not buried in a proper manner, he will come back in real life to haunt the living family members.

Knowledge levels about EVD and MVD were different across different socio-demographic and other study variables. Being educated beyond primary level was the most significant predictor of awareness towards filoviruses. For example, people who have attained the secondary level of education were more likely to be knowledgeable about filoviruses as opposed to those who did not attain any formal education (OR = 3.6; 2.1–6.1). These odds were even higher for individuals who attained education beyond secondary school. This is correct because education is a key determinant of knowledge especially concerning health and health seeking behaviors and it has been found to influence people's knowledge about EVD in Nigeria [18]. Education was still significant even after controlling for other variables such as sex and age. Males were more likely to be knowledgeable about filoviruses than females, possibly related to education because in many African societies, men are more apt to be more educated than women. However, males were still significant even after controlling for formal education meaning there are other contributing intrinsic factors. For example, information access may be more to men than women. People from MVD affected communities were more knowledgeable than people from EVD affected communities. Although the two communities come from two different tribes and live distant from each other one in the Western Uganda and another in Central Uganda, we do not find any plausible explanation as to why there should be a difference in the level of knowledge. This may, however, be influenced by the impact of the two diseases EVD being more pathogenic, causes more socio-cultural disruption leading to myths and misconceptions hence negative attitude and less knowledge about it. These factors were different from those reported by Iliyasu *et al.* (2015) [18] where the predictors of knowledge about EVD were being a health worker, being afraid about Ebola, and willingness to modify behavior. However, in another study in Nigeria, it was reported that education was a critical predictor of knowledge [44, 45], also indicated by comparing literacy rates of Uganda and West African countries. The countries that were affected by Ebola in West Africa have lower literacy rates compared to Uganda [46]. This could partly explain why people could not comprehend EVD as a disease in West Africa hence the increased transmission as compared to Uganda where people are more educated and experience low transmission rates of EVD to the extent of getting only one case in 2011 in Luweero district.

We explored gender disparities in this study using a proportional piling technique. Although many studies show that VHFs tend to affect more females than males because of their gender roles [47, 48], our study revealed that almost all men and women are affected

equally (S5 Fig). However, from the FGDs, men were indicated as more likely to be index cases than women because of their risky behavior and gender roles such as hunting, clearing land for agriculture and going into the forest for several activities. As the outbreak progresses, women tend to be more likely to be affected since they are more into caring for the sick hence have higher chances of being infected.

These results from our study may not be generalized to the communities in the whole of Uganda. Studied communities were selected purposively because of their previous experience with Ebola and Marburg outbreaks. Communities that have experienced outbreaks are more likely to have received education through social mobilizations that happened during outbreaks, and hence appear to be more knowledgeable than other communities that have not experienced outbreaks. Another limitation of this study could be possibly biased responses drawing from outbreak experiences. Probably the answers and knowledge assessment outcomes would be different if the same study is done in an entirely naïve population.

Conclusion

In conclusion, the study revealed that communities in Uganda that had been affected by filovirus outbreaks are slightly knowledgeable and have a good attitude towards control and prevention of EVD. Formal education is a significant predictor of knowledge and attitude towards filoviruses. Communities could identify the suspect cases and are aware of the modes of transmission, and they suggest sensitization as the best approach for control of filovirus outbreaks. Although Uganda health sector has developed preparedness plans to respond to filovirus outbreaks, the level of knowledge about filoviruses is still below average and needs to be improved. The public health sector could enhance communities' knowledge and attitude by supplying more educational materials and conducting health education for epidemic preparedness and using appropriate communication channels as proposed by the communities.

Supporting information

S1 Questionnaire. Questionnaire that was used to collect quantitative data.
(PDF)

S1 FGD guide. Focused group discussion guide that was used to collect qualitative data.
(PDF)

S1 Fig. This is a picture showing how participants in one of the FGDs ranked the most important clinical signs of Ebola Virus disease. The clinical signs are written in one of the local languages in Uganda, Luganda.
(TIF)

S2 Fig. This picture shows proportional piling technique where participants used 100 grains of beans to distribute them according to what they think is most important in transmitting Ebola Virus disease. Words are written in the local language, Luganda.
(TIF)

S3 Fig. The picture shows pairwise ranking technique where participants listed and compared the possible causes of filovirus outbreaks among themselves to come up with a rank of the most important cause. Causes were listed in both rows and columns in the local language.
(TIF)

S4 Fig. The Receiver Operating Curve(ROC) that was used to assess the model for predictors of knowledge towards Ebola and Marburg virus diseases.
(TIF)

S5 Fig. This picture shows proportional piling of 100 grains of beans to determine which gender is affected most by filovirus outbreaks.
(TIF)

S1 Table. Themes and categories generated from focused group discussions by conventional content analysis technique about People's knowledge and attitude towards Ebola and Marburg virus diseases.
(DOCX)

S2 Table. Results of pairwise ranking technique applied on risk factors/causes of Ebola and Marburg virus diseases.
(DOCX)

S3 Table. An alternative model to the logistic regression model if no categorisation of knowledge is done. The predictors of knowledge score are the same as those in the logistic regression.
(XLSX)

S1 Data. Quantitative data set.
(XLSX)

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Author Contributions

Conceptualization: Luke Nyakarahuka, Eystein Skjerve, Stephen Balinandi, Trevor Shoemaker, Clovice Kankya.

Data curation: Luke Nyakarahuka, Eystein Skjerve, Daisy Nabadda, Chisoni Mumba, Stephen Balinandi, Trevor Shoemaker, Clovice Kankya.

Formal analysis: Luke Nyakarahuka, Eystein Skjerve, Daisy Nabadda, Doreen Chilolo Sitali.

Funding acquisition: Eystein Skjerve, Trevor Shoemaker, Clovice Kankya.

Investigation: Luke Nyakarahuka, Daisy Nabadda, Trevor Shoemaker, Clovice Kankya.

Methodology: Luke Nyakarahuka, Daisy Nabadda, Doreen Chilolo Sitali, Chisoni Mumba, Trevor Shoemaker, Clovice Kankya.

Project administration: Eystein Skjerve, Trevor Shoemaker, Clovice Kankya.

Resources: Eystein Skjerve, Stephen Balinandi, Trevor Shoemaker, Clovice Kankya.

Software: Eystein Skjerve.

Supervision: Eystein Skjerve, Frank N. Mwiine, Julius J. Lutwama, Clovice Kankya.

Validation: Luke Nyakarahuka, Eystein Skjerve, Chisoni Mumba, Frank N. Mwiine, Julius J. Lutwama, Clovice Kankya.

Visualization: Luke Nyakarahuka, Chisoni Mumba, Julius J. Lutwama, Clovice Kankya.

Writing – original draft: Luke Nyakarahuka.

Writing – review & editing: Luke Nyakarahuka, Eystein Skjerve, Daisy Nabadda, Doreen Chililo Sitali, Chisoni Mumba, Frank N. Mwiine, Julius J. Lutwama, Stephen Balinandi, Trevor Shoemaker, Clovice Kankya.

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PAPER IV

A Retrospective Cohort Study of Seroprevalence of Ebola and Marburg viruses in humans from two different ecological zones in Uganda

Luke Nyakarahuka[1 4], Ilana Schafer[2], Stephen Balinandi[3], Sophia Mulei[1], Alex Tumusiime[3], Jackson Kyondo[1], Barbara Knust[2], Julius Lutwama[1], and Trevor Shoemaker[3]

[1] Viral Hemorrhagic Fevers Program, Uganda Virus Research Institute, Entebbe, Uganda;

[2] Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, USA;

[3]Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Entebbe, Uganda;

[4] Norwegian University of Life Sciences (NMBU), Oslo Norway.

Abstract

Uganda has experienced five Ebola Virus Disease(EVD) outbreaks and three Marburg Virus disease(MVD) outbreaks between 2000 and 2017. We investigated the seroprevalence and risk factors for Marburg virus and ebolaviruses infections in gold mining communities around Kitaka mine in Western Uganda compared them to non-mining communities in Central Uganda

Human blood samples were collected from three groups in Western Uganda (miners, family members of miners, non-miners living 30km away from Kitaka mine) and one group in Central Uganda far away from the Kitaka mine as controls. ELISA technique was used to analyze sample, detecting antibodies against Marburg virus and ebolaviruses.

The filovirus IgG antibody seropositivity for all particicpants was 2.6% (19/724) of which 2.5% (18/724) was to *Sudan ebolavirus,* 0.1% (1/724) was to *Bundibugyo ebolavirus*, and 0.1 %(1/724) to Marburg virus. One individual had IgG antibodies reactive to both *Sudan ebolavirus* and *Bundibugyo ebolavirus*. The risk factors for filovirus infection identified included mining (AOR=3.4, 1.3-8.5), male sex (3.1, 1.01 - 9.5), going inside mines (3.1, 1.2 - 8.2), cleaning corpses (3.1, 1.04 - 9.1) and contact with filovirus suspect cases (3.9, 1.04 - 14.5).

These findings indicate that filovirus outbreaks may go undetected in Uganda and and people involved in artisan gold mining or living close to caves inhabited by bats are more likely to be

exposed to filovirus infection. This calls for active surveillance in known high-risk areas for early detection and response to prevent filovirus epidemics.

Introduction

Viruses in the genuses *Ebolavirus* and *Marburgvirus* belong to the family *Filoviridae,* and cause classical viral hemorrhagic fevers (VHFs) in humans, which are associated with high morbidity and mortality and pose a serious threat to human and animal populations in endemic countries. Uganda has reported eight filovirus outbreaks from 2000 to 2017, including five Ebola virus disease (EVD) outbreaks and three Marburg virus disease (MVD) outbreaks (Nyakarahuka et al., 2016). The first EVD outbreak in Uganda was reported in 2000 in Gulu district, Northern Uganda (Oyok et al., 2001). Subsequently, four more outbreaks of EVD have been reported (Nyakarahuka et al., 2016) and two of these have been reported in one district of Luweero in Central Uganda (Shoemaker et al., 2012, Mbonye et al., 2012, Albarino et al., 2013). In Ibanda and neighboring Kamwenge districts alone, there have been two documented outbreaks of MVD (Knust et al., 2015, Adjemian et al., 2011). The first occurred in 2007 with four confirmed cases and two deaths (CFR=50%) (Adjemian et al., 2011). The four confirmed cases were either gold miners or persons associated with gold mining in Kitaka mine along the Ibanda-Kamwenge district border. Previous studies have found bats of species *Rousettus aegyptiacus* to be the known reservoir for Marburg virus (Amman et al., 2012, Amman et al., 2014, Towner et al., 2009). This species of bat has been known to inhabit Kitaka mine in Ibanda district as well as in Maramagambo "*python cave"* in the neighboring Rubirizi district. Two tourists visiting python cave were infected with Marburg virus in 2008 with one case being fatal (Centers for Disease and Prevention, 2009, Timen et al., 2009). In 2012, the second outbreak of MVD was identified in Ibanda district (Knust et al., 2015). The retrospective case investigation traced the outbreak's origin to villages near Kitaka mines.

Despite multiple filovirus infections having been confirmed in Uganda, the real burden of these hemorrhagic fever virus infections is not known. For example, Marburg virus outbreaks have been detected from Ibanda and Kamwenge districts near mining communities. However, our understanding of the possible linkages between artisanal gold mining activities and Marburg virus infection is poor. We wanted to better understand the possible link between artisanal gold mining activities and the transmission of Marburg virus. We performed a retrospective cohort study, in miners, their close contacts, and persons living close to Kitaka mine in Ibanda and Kamwenge districts to determine a risk factords for filovirus seropositivity associated with

kitaka mine. Other potential risk factors for Marburg and Ebola virus infection were also investigated. The results of the sampled people were compared between those in Kamwenge and Ibanda districts and those in Luweero district where there are no mining operations and which is situated in a different ecological zone. We hypothesized that miners and their close contacts/family members from Ibanda and Kamwenge districts are at greater risk of Marburg virus infection than the general population of Ibanda/Kamwenge districts and the population in Luweero district. Non-miners who live near Kitaka mine may also be at greater risk of exposure to Marburg virus than the general Ibanda/Kamwenge population and the population in Luweero district. This information will give insight into better control and prevention measures for future outbreaks of filoviruses in Uganda. This information will give insight into better control and prevention measures for future outbreaks of filoviruses in Uganda.

Methods

Study site, design, and population

Study participants were sampled from Ibanda, Kamwenge and Luweero districts (Figure 1). The bat-inhabited Kitaka mines are located within the boundary of Ibanda and Kamwenge districts. The mine is within Kasyoha-Kitomi Forest Reserve, which acts as a buffer zone between Queen Elizabeth National Park and human settlements. This Forest Reserve is shared by the two districts of Kamwenge and Ibanda and located within the Albertine region with known high biodiversity. The study was focused around Kitaka mine, a known habitat of Marburg virus reservoir bats (*Rousettus aegyptiacus).* Abandoned gold mines and caves, may also have other wildlife that are potential reservoirs of filoviruses. Communities that live in and around this reservoir were considered as the "Exposed" group for our study.

A control group group in Luweero district was chosen because it is in the Central region of the country far from Kitaka mines and we hypothesized that *Rousettus aegyptiacus* bats may not inhabit this region due to lack of suitable habitat, and therefore inhabitants would mostly likely not be exposed to Marburg virus. It is not forested, has no National Parks or mining activities and therefore does not provide the correct habitat for the bats known to harbor Marburg virus (*Rousettus aegyptiacus)*. Also, Luweero has experienced two EVD outbreaks in recent years with no known MVD outbreaks. Individuals living in Luweero district were considered the "Un-exposed," or overall control group.

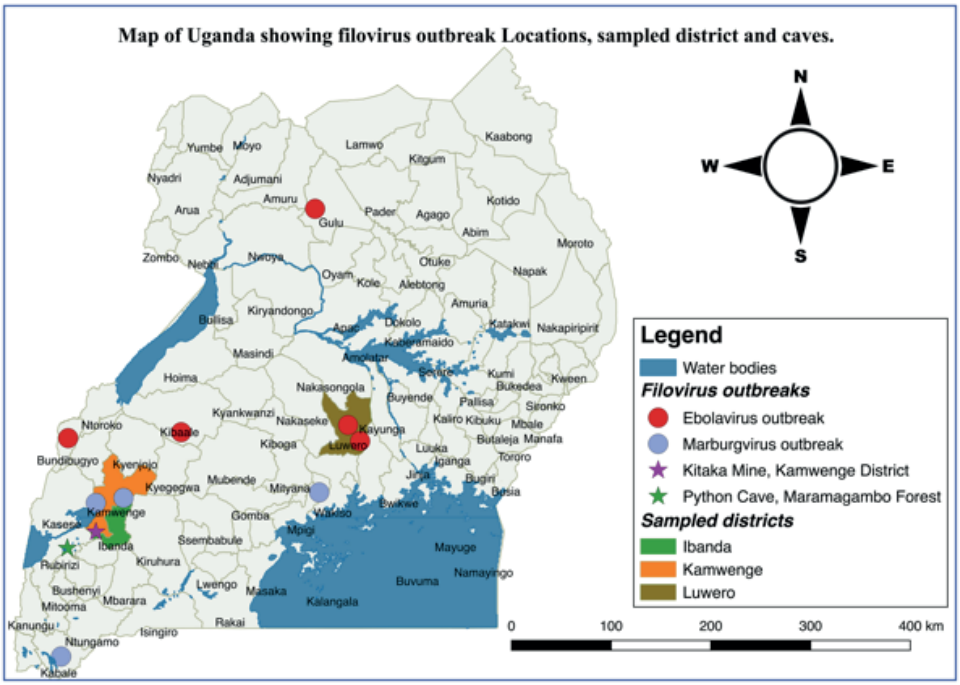

Figure 1: Reported filovirus outbreaks and studied districts

Sampling procedure, inclusion and exclusion criteria

We sampled four groups of individuals that included; 1) Miners and persons that have worked in the Kitaka mines from 2007 to present, 2) Members of the household or family housing compound of a miner during the time period that the miner was actively working in Kitaka mines, 3) Members of households that reside within 50 km radius from any open mining site associated with Kitaka mines, and that were not included in above groups 1 or 2, and 4) residents of Luwero district. For groups 1 and 2, a purposive sampling procedure was used with a snowball approach. Participants were questioned to determine those currently working or those who used to work in Kitaka mines. The discovered miners were further questioned to identify additional miners or ex-miners. All discovered miners and their family members who were willing to participate were included in the study. For groups 3 and 4, random sampling of villages was employed. Once a village was selected, the investigators traveled to the location of the main trading post at the village's center, and participants were chosen following the EPI method (Bennett et al., 1991). Participants that consented to inclusion in the study were interviewed to complete a risk factor questionnaire, and provided their answers verbally. One

blood sample (minimum 4ml) was collected from each participant for serological testing for filovirus IgG by ELISA at Uganda Virus Research Institute (UVRI)/Centers for Disease Control (CDC) VHF laboratory. The total sample size was determined to be 500 (200 unexposed and 300 unexposed); estimating a 15% prevalence of filovirus infection in the exposed groups versus 5% prevalence in the unexposed group, with a 95% confidence interval, 80% power, and a ratio of 2 unexposed people to each exposed person..

Data management and analysis.

Data was entered in *Epinfo 7* and analyzed using STATA (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). We computed Odds Ratios (OR) that provides a reasonable estimation of the Risk Ratio (RR) since the outcome in the exposed population is less than 10% (Viera, 2008). We controlled for confounding by adjusting for sex, age, occupation, and education level by computing the adjusted odds ratio (AOR).

Laboratory Analysis

All the samples collected were tested by Enzyme-Linked Immunosorbent Assay (ELISA), which was validated by US Centers for Disease Control and Prevention (CDC) on known positive and negative human samples with a sensitivity of more than 90% and specificity of more than 90% (Ksiazek et al., 1999). Briefly a gamma-irradiated lysate of Vero cells infected with either *Sudan ebolavirus*, *Bundibugyo ebolavirus*, *Zaire ebolavirus*, or Marburg virus was used as positive antigen whereas the negative or control antigen had uninfected Vero cells. 100µl of positive Antigen diluted in PBS (Marburg Ag 1:3000 and Ebola Ag 1:2000 Dilutions) was applied on the upper half of the solid phase of a polyvinyl chloride microtiter plate and the lower half coated with 100µl of negative/control antigen in PBS then incubated at 4˚C overnight. Unbound antigen was removed from the well by washing three times with PBS-Tween. Samples were diluted 1:100 and 4-fold through 1:6400 in 5% skimmed milk in PBS-Tween and allowed to bind to the antigen. After washing, an anti-human IgG conjugated to horseradish peroxidase (HRPO) was added and allowed to bind. The plates were washed and the substrate ABTS (2.2'-Axinobis 3-ethylbenzothiazoline-6-sulfonic acid-diammonium salt) was added which in the presence of HRPO and hydrogen peroxide, is converted from a colorless liquid to an intense green color with a maximum light absorption at 410 nm. The amount of color developed is proportional to the amount of IgG antibodies which has bound to the antigen on the solid phase. OD values at 410nm were recorded on a microplate spectrophotometer. The OD value of the control antigen-coated well was subtracted from its

corresponding viral antigen-coated well to yield adjusted OD value. A sample was considered positive when the adjusted OD value of either the 1:400, 1:1600 or 1:6400 dilution was greater than 0.2 and the sum OD value was greater than 0.95. A panel of 1 or 2 negative control sera and 2 or 3 positive control sera were run each time the assay was used.

Ethical considerations

Approval was obtained from the Uganda Ministry of Health National Taskforce (NTF) on Ebola and Marburg virus outbreaks to conduct this study as a follow up to the 2012 Marburg outbreak. Additionally, approval from CDC was obtained through a determination that the investigations were a follow-up to the MVD outbreak and was classified as non-research. Approval (No. HS 1538) from the UVRI Research and Ethics Committee and the National Council of Science and Technology was obtained. Written consent was obtained from each study participant and for those below 18 years, consent was provided by their parent or guardian.

Results

Overall, we sampled 724 individuals, 433 (59.8%) from the exposed region in Western Uganda (Ibanda and Kamwenge districts) and 291 (40.2%) from the unexposed district of Luweero in Central Uganda. The mean age was 36.3 (SD=14.8, 95%CI=35.2-37.4), the median age was 33.0 (3-82). 85. 6% (620/724) of the sampled people were ≥ 20 years and 54.1% (391/724) were male. 71.6% of participants had primary school education or less, and 67.7% were farmers followed by miners at 22.2%. Other practices identified that could be risk factors for filovirus infection included going inside mines (19.1%), contacts with bats in the mines (34.7%), owning domestic animals (77.8%), hunting (3.9%), eating bushmeat (47.9%), cleaning dead bodies at funerals (12.5%), going to the forest frequently (66.3%) and having bats in the house (56.2%).

Table 1 shows that miners and their family members are at higher risk of infection with *Sudan ebolavirus* than unexposed cohort from Luweero district (RR=3.9, 95% CI 1.1-13.7). In total, 2.6% (19/724) individuals tested had IgG antibodies against filoviruses. Eighteen individuals had *Sudan ebolavirus* IgG antibodies seropositivity (2.5%, 18/724), and one person had IgG antibody seropositivity to both *Sudan ebolavirus* and *Bundibugyo ebolavirus* (0.1%, 1/724). One person had IgG antibody against Marburg virus (0.1%, 1/724). No individuals had IgG antibody against *Zaire ebolavirus*.

Other risk factors investigated are shown in Table 2. These include male sex (AOR=3.1, 1.01 - 9.5), going inside mines (AOR=3.1, 1.2 - 8.2), cleaning corpses (AOR=3.1, 1.04 - 9.1) and contact with EVD/MVD suspects (AOR=3.9, 1.04 - 14.5). Frequent travels (once a month) outside a persons' home district was shown to be protective (AOR=0.3, 0.1-0.7).

Table 1: Summary of study groups and corresponding seroprevelances and risk ratios.

Study Cohorts	Number sampled	Marburg virus seroprevalence (%)	*Sudan ebolavirus* (SUDV) seroprevalence (%)	SUDV Risk Ratio (95%CI)
Unexposed (Luweero district)	291	0	3 (1.1%)	Reference[a]
Miners only	161	1 (0.62%)	8 (4.96%)	4.8 (1.3 - 17.9) *
Family Member of miner	138	0	4 (2.9%)	2.8(0.64-12.4)
Miners and Family members	299	1 (0.33%)	12 (4.0%)	3.9(1.1-13.7) *
Non-miners within 30km away from Kitaka mine	134	0	3 (2.2%)	2.2(0.44-10.6)
All exposure groups from Western Uganda	433	1 (0.23%)	15 (3.5%)	3.4 (0.98-11.5)

[a] All other groups were compared to the unexposed group as control

*Statistically significant

Table 2: Risk factors for filovirus seropositivity

Variable	Category	Total Number- N	Filovirus IgG Seropositive	Filovirus IgG Seronegative	Crude RR (95% CI)	[a]Adjusted RR(95%CI)
Total participants		724	19(2.6%)	705(97.4%)		
Age (years)	<20	104(14.4%)	1(0.96%)	103(99.04%)	Ref	
	> 20	620(85.6%)	18(2.9%)	602(85.4%)	3.0(0.4-22.4)	1.9(0.2-14.7)
Gender	Female	333(45.9%)	4(1.2%)	329(98.8%)	Ref	
	Male	391(54.1%)	15(3.8%)	376(96.2%)	3.3(1.1-9.9)*	3.1(1.01 - 9.5)*
Education	Never	122(16.9%)	5(4.1%)	117(95.9%)	Ref	
	Primary	397(54.8%)	10(2.5%)	387(97.5%)	0.6(0.2-1.8)	0.4(0.1-1.3)
	Secondary	186(25.7%)	4(2.2%)	182(97.9%)	0.5(0.1-1.9)	0.3(0.1-1.4)
	Tertiary	19(2.6%)	0(0%)	19(100%)	1	
District	Luweero	291(40.2%)	3(1.1%)	288(98.9%)	Ref	
	Ibanda	244(33.7%)	9(3.7%)	235(96.3%)	3.7(0.9-13.7)	2.4(0.6-10.2)
	Kamwenge	189(26.1%)	7(3.7%)	182(96.3%)	3.7(0.9-14.5)	2.4(0.6-10.7)
Famer	No	227(32.3%)	5(2.2%)	222(97.8%)	ref	
	Yes	475(67.7%)	14(2.9%)	461(97.5%)	1.3(0.5-3.8)	1.3(0.4-3.6)
Go inside mines	No	586(80.9%)	10(1.7%)	576(98.3%)	Ref	
	Yes	138(19.1%)	9(6.5%)	129(93.5%)	4.0(1.6-10.1)*	3.1(1.2 - 8.2)*
Contact with bats in mines	No	115(65.3%)	4(3.5%)	111(96.5%)	Ref	
	Yes	61(34.7%)	5(8.2%)	56(91.8%)	2.5(0.6-9.6)	1.9(0.5-7.4)
Own Domestic animals	No	162(22.4%)	3(1.8%)	159(98.2%)	Ref	
	Yes	562(77.8%)	16(2.8%)	546(97.2%)	1.6(0.5-5.4)	1.3(0.4-4.8)
Contact with Animals	No	163(22.5%)	2(1.2%)	161(22.8%)	Ref	
	Yes	561(77.5%)	17(3.1%)	544(96.9%)	2.5(0.6-11)	3.7(0.4-36.3)
Hunting	No	596(82.3%)	14(2.3%)	582(97.7%)	Ref	
	Yes	128(17.7%)	5(3.9%)	123(96.1%)	1.7(0.6-4.8)	1.1(0.4-3.4)
Contact with dead animals	No	637(90.6%)	16(2.5%)	621(97.5%)	Ref	
	Yes	66(9.4%)	3(4.5%)	63(95.5%)	1.8(0.5-6.5)	1.4(0.4-4.9)
	No	377(52.1%)	6(1.6%)	371(98.4%)	Ref	

Eat bush meat						
	Yes	347(47.9%)	13(3.7%)	334(96.3%)	2.4(0.9-6.4)	2.0(0.7-5.6)
Cleaning of dead body	No	602(87.5%)	12(2%)	590(98.0%)	Ref	
	Yes	86(12.5%)	5(5.8%)	81(94.2%)	3.0(1.1-8.3)*	3.1(1.04 - 9.1)*
MVD reported in the village	No	574(79.3%)	11(1.9%)	563(98.1)	Ref	
	Yes	150(20.7%)	8(5.3%)	142(94.7%)	2.9(1.1-7.3)*	2.2(0.8-6.2)
Contact with EVD/MVD suspects	No	693(95.7%)	16(2.3%)	677(97.7%)	Ref	
	Yes	31(4.3%)	3(9.7%)	28(90.3%)	4.5(1.2-16.5)*	3.9(1.04 - 14.5)*
Frequently travels	No	119(16.4%)	7(5.9%)	112(94.1%)	Ref	
	Yes	605(83.6%)	12(2%)	593(98.0%)	0.3(0.1-0.8)*	0.3(0.1-0.7)*
Go to the Forest frequently	No	244(33.7%)	4(1.6%)	240(98.4%)	Ref	
	Yes	480(66.3%)	15(3.1%)	465(96.9%)	1.9(0.6-5.9)	2.(0.7-6.1)
Wash fruits before eating	No	430(61.4%)	13(3.0)	417(96.9%)	Ref	
	Yes	270(38.6%)	6(2.2%)	264(97.8%)	0.7(0.3-1.9)	0.9(0.3-2.4)
Reported of bats in the house	No	317(43.8%)	4(1.3%)	313(98.7%)	Ref	
	Yes	407(56.2%)	15(3.7%)	392(96.3%)	2.9(0.9.9.1)*	2.5(0.8-7.8)

*** Statistically significant**

[a] Adjusted for gender, age, education level, and occupation

Discussion

We report for the first time seroprevalence of filoviruses in Uganda in apparently healthy individuals. Our findings point to the hypothesis that filovirus infections may occur in Sub-Saharan African countries and go undetected by the health care systems. Also our findings suggest that people who are involved in artisanal gold mining and live close to caves inhabited by bats are at higher risk of infection with filoviruses. This could possibly lead to large epidemics as was seen in West Africa (2014). Here, we report 19 individuals that were seropositive for IgG antibodies against filoviruses representing 2.6% of the people tested. Out of these 19, 18 were seropositive for *Sudan ebolavirus,* and one was seropositive for Marburg virus. Of the 18 seropositive for *Sudan ebolavirus*, one was also seropositive for *Bundibugyo ebolavirus*, likely a cross-reactivity rather than representing previous exposure to both *Ebolavirus* species, as has been described previously. This is slightly lower at 3% and 8% for pooled prevalences reported in meta-analyses of seroprevalence of Ebola virus performed in other parts of the world (Nyakarahuka et al., 2016, Bower and Glynn, 2017). Also, following the West Africa outbreak, reports of asymptomatic infection in West African populations has been suggested (Glynn et al., 2017, Richardson et al., 2016). Our study also reported a lower seroprevalence of Ebola virus than that reported in other studies in neighbouring Democratic Republic of Congo (DRC) (Busico et al., 1999, Becquart et al., 2010, Heymann et al., 1980, Commission, 1978, Nkoghe et al., 2011, Van der Groen and Pattyn, 1979), Central African Republic, Gabon (Allela et al., 2005, Heffernan et al., 2005, Lahm et al., 2007, Georges et al., 1999, Bertherat et al., 1999), Sudan (Baron et al., 1983), Madagascar (Mathiot et al., 1989), Liberia (Meunier et al., 1986) and Cameroon (Bouree and Bergmann, 1983, Paix et al., 1988), but showed higher seroprevalence than that reported in Nigeria (Tomori et al., 1988), Germany (Becker et al., 1992) and Kenya (Johnson et al., 1983). Only one Marburg virus seropositive person was confirmed in our study and this was much lower than has been reported in other studies (Van der Waals et al., 1986, Gonzalez et al., 1989, Johnson et al., 1983, Mathiot et al., 1989, Becker et al., 1992, Johnson et al., 1993a, Johnson et al., 1993b).

We also report for the first time seroprevalence of *Bundibugyo ebolavirus* in one individual, while no individuals showed seroprevelance for Ebola Zaire virus. These variations in seroprevalence could be due to differences in filovirus ELISA testing protocols. The test, developed by CDC that was used in this study has been shown to be more specific than other filovirus serological tests used in previous filovirus seroporvelance studies (Ksiazek et al.,

1999). This serological test was developed and validated by US Centres for Disease Control and Prevention (CDC) on known positive and negative human samples with a sensitivity >90% and specificity of >90%. However, we still see serological cross-reactivity within filovirus species even with this test. In this study, for example, one *Sudan ebolavirus* IgG-positive blood sample was also positive for *Bundibugyo ebolavirus* IgG antibodies. This cross-reactivity has been reported in several other studies (Macneil et al., 2011, Natesan et al., 2016).

Geographical differences that favor a filovirus reservoir in different countries in Africa could explain the variation in seroprevalences. We cannot clearly explain why Marburg virus seroprevalence is lower than that of Ebola, but this is consistent with other studies that have done serosurveys where the two pathogens have been tested. One of the explanations could be that the antibodies for Marburg virus are not as long-lasting compared to those of Ebola virus, but this needs to be explored in further studies.

We also see a higher seroprevalence in our exposed group at 3.7% (16/433) compared to unexposed group at 1.1% (3/291) although this difference is confounded by the fact that in our exposed study participants, the majority are miners and they had an even higher prevalence of 5.6% compared to non-miner (1.8%), AOR=3.4 (1.3-8.5). As has been reported before, the Kitaka mines where the exposed population is centered is inhabited by bats of species *Rousettus aegyptiacus* that are the known reservoirs for Marburg virus (Adjemian et al., 2011, Amman et al., 2012, Amman et al., 2014, Towner et al., 2009). We expected a higher seroprevalence against Marburg virus than Ebola virus, but the opposite was observed with Ebola virus seroprevelance being higher than Marburg virus. Whereas it has been confirmed during previous investigations that bats occupying the mines are actively infected with Marburg virus and had been associated with two MVD outbreaks (Adjemian et al., 2011, Knust et al., 2015), no outbreak of EVD has been reported in this region. It was therefore surprising to find higher seroprevalence to *Sudan ebolavirus* instead of the expected Marburg virus. We hypothesize that there may be a reservoir for *Sudan ebolavirus* or another closely related filovirus in Kitaka mines and/or inhabiting the area around the Kasyoho-Kitomi reserve ecosystems to which these individuals were exposed, especially the gold miners. This area is near Queen Elizabeth National Park, and so there is a possibility of having an unknown reservoir of Ebola virus in the game reserve that has not been previously identified. This is in contrast to our unexposed groups from Central Uganda, Luweero district where only three people were identified as being seropositive for *Sudan ebolavirus*, and none was positive for any other species of filoviruses.

The only positive MVD case was in a miner from the exposed region, and he did not show seroprevelance for any other filovirus species.

Our findings are consistent with what was reported in another filovirus serological study by Nkoghe *et al.*(2011) in rural Cameroon and Gabonese populations where the prevalence of Ebola virus was higher in populations near forests (Nkoghe et al., 2011, Becquart et al., 2010, Bouree and Bergmann, 1983). Although no other risk factors were identified in Gabonese study, we find in our study that being a miner is highly associated with being seropositive for *Sudan ebolavirus.* Although we are reporting seroprevalence of *Sudan ebolavirus,* the risk factors for all species of *Ebolavirus* are thought to be the same. Another study in the Gabon found that pygmies, who are forest dwellers, had a higher percentage of ebolavirus seroprevalence than other populations at 7.02% compared to non-pygmies (4.2%) (Gonzalez et al., 2000). This further indicates that communities that live in the forested areas, like the ones we studied in Uganda are at higher risk of infection with filoviruses compared to those living in more developed or non-forested areas. Forested areas tend to have a greater abundance of fruiting trees that provide food to the fruits bats, the hypothesized reservoirs of Ebola virus. However, in this study, going into the forest was not shown to be a risk factor for individuals being seropositive for filoviruses.

Gold mining has been previously described as a risk factor for Marburg virus infection in a study in DRC (Bausch et al., 2003) with OR=13.9, 95%CI;3.1-62.1 but not for Ebola virus. We report artisanal mining and going inside the mines as risk factors for being seropositive for filovirus in Western Uganda (AOR=3.4, 1.3-8.5), although the very first cases of Ebola virus were reported in mining communities in DRC in 1976. Other factors that we identified as risk factors include being a miner or a family member of a miner. Since filoviruses are spread by contact when miners fall sick, they are primarily taken care of by family members, and hence are likely to be at greater risk of acquiring the infection. The four cases we found in the unexposed group could be due to travel and migration from high-risk areas, but may also be due to the movement of reservoirs such as bats that are known to travel long distances hence spreading the infection. Ebola seropositivity has before been reported in a grassland savanna-like ecosystem in Nigeria similar to the grassland savana ecosystem of Luweero where the four (4) seropositive cases came from (Tomori et al., 1988). However, frequent travels outside high-risk areas were protective (0.3; 0.1-0.7). This is because people who frequently travel away from exposed areas are less exposed to the putative reservoir. Being male was associated with a high risk of being seropositive (3.1;1.01-9.5) compared to being female, partly because

men are more likely to be miners and go inside the mines and the forests hence acquire infection and be index cases which bring the infection to the rest of the family members. Participating in a funeral, especially cleaning or preparing the dead body, was highly associated with being seropositive for a filovirus. This has been widely reported in outbreaks of filoviruses as burials and funeral rites amplify these outbreaks. Unlike the study by Nkonghe et al(2011). in Gabon, receiving injections was not a risk factor in this study simply because of increased infection control in hospitals in Uganda. Contact with EVD/MVD suspect was very important as a predictor of seropositivity with a filovirus and has been reported in a partial meta-analysis done on the risk of Ebola transmissions (Brainard et al., 2015).

These findings should not be over interpreted as the study could be biased towards high-risk groups. In addition, testing for filoviruses using serological tests is still highly debated because of reported cross-reactivity and variation in testing methods for the different serological tests used in previous studies.

We conclude that filovirus infections do occur and may go undetected by the health care system. Also, people who live near caves inhabited by fruit bats are at higher risk of filovirus infection compared to populations leaving far away from these caves. Increased surveillance is critical in averting future widespread and devastating filovirus epidemics.

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PAPER V

Seroepidemiological Study of Ebolaviruses in Domestic Animals from Africa: Detection of IgG antibodies against Ebolaviruses in Goats from Uganda, Ivory Coast and the Democratic Republic of Congo

Luke Nyakarahuka*[1,2,3,4], Susanne Koehler[2], Ulla Thiesen[2], Clovice Kankya[3], Terence Odoch[3], Frank N. Mwiine[3], Julius J. Lutwama[4], Eystein Skjerve[1], Fabian Leendertz[2]

1. Norwegian University of Life Sciences, Department of Food Safety and Infection Biology Oslo Norway
2. Robert Koch Institute, Project Group 3, Epidemiology of Highly Pathogenic Microorganisms, Berlin Germany
3. Makerere University, Department of Biosecurity, Ecosystems and Veterinary Public Health, College of Veterinary Medicine, Animal Resources and Biosecurity, Kampala Uganda
4. Uganda Virus Research Institute, Department of Arboviruses, Emerging and Re-emerging Disease Entebbe Uganda.

*Corresponding Author.

Abstract

The search for the natural reservoir of Ebolaviruses is still ongoing, and there has not been a clearly described primary reservoir. Domestic animals in Africa live at the interface between wildlife and human, hence making them good candidates for investigation as possible source of filovirus spill-overs into the human population. We used serological approaches to investigate domestic goats as potential reservoirs and source of infection to humans in Uganda, Democratic Republic of Congo and Ivory Coast. Many serological studies have been conducted, but with contradicting results due to cross reactivity and interpretation difficulties. Accordingly, our preliminary result using ELISA and classical Western blot data showed reactivity to GP, NP and VP40. To put these results into perspective all samples were tested independently again with a Luminex assay which showed reactivity to GP, NP and VP40 from *Zaire ebolavirus, Sudan ebolavirus, Bundibugyo ebolavirus* and *Reston ebolavirus.* Our findings indicate that goats from three countries have antibodies against recombinant ebolavirus antigens GP, NP and VP40. We recommend further research to rule out the possibility of cross-reactivity with other Sub-Saharan pathogens.

Introduction

Ebola Virus Disease (EVD) is caused by one of the *Ebolavirus* species in the family *Filoviridae*. So far five species have been described which include *Zaire ebolavirus, Sudan*

ebolavirus, Bundibugyo ebolavirus, Reston ebolavirus and *Taï Forest ebolavirus*. Outbreaks of EVD do not only cause mortality and morbidity of humans and animals but also have devastating socio-cultural and economic consequences as was the case in West Africa. In 2014, an outbreak of EVD in West Africa started in Guinea spread to the neighboring countries of Liberia, Sierra Leone and later to other parts of the world including Nigeria, United States, United Kingdom, Spain, Italy, Senegal and Mali with a recorded number of suspected, probable and confirmed cases being 28,616 and recorded deaths at 11,310 (Team, 2016). Previous outbreaks of EVD had been reported in other countries in Africa which include Democratic Republic of Congo (DRC) (1978b, Heymann et al., 1980, Khan et al., 1999, Leroy et al., 2009, Grard et al., 2011, Albarino et al., 2013, Maganga et al., 2014), South Sudan (1978a, Baron et al., 1983, Onyango et al., 2007), Gabon (Amblard et al., 1997, Georges et al., 1999, Milleliri et al., 2004, Nkoghe et al., 2005), Republic of Congo (Rouquet et al., 2005, Boumandouki et al., 2005, Nkoghe et al., 2011) and Uganda (Okware et al., 2002, Wamala et al., 2010, Shoemaker et al., 2012, Albarino et al., 2013).

To control and prevent future outbreaks, there is need to understand the epidemiology of EVD, especially the source of infection for index cases in these outbreaks and the natural reservoir for Ebola virus. There has been an effort by the scientific community in this direction following previous outbreaks, but no true reservoir or direct source of infection has been described. There is no conclusive evidence that bats are reservoirs of Ebola virus and hence potential sources of infection to humans (Leendertz et al., 2016). However, cave-dwelling Egyptian fruit bats (*Rousettus aegyptiacus*) have been found to be reservoirs of another related filovirus, Marburg virus in Uganda (Amman et al., 2012, Amman et al., 2014, Towner et al., 2009). Non-human primates are known to be infected by *ebolaviruses* but seem to succumb to the infection hence unlikely to be true reservoirs (Rouquet et al., 2005, Leroy et al., 2004). Other wildlife species apart from bats and non-human primates that have tested positive include a duiker by PCR (Rouquet et al., 2005) and domestic dogs by serology (Allela et al., 2005). However serological results should not be over interpreted as there tends to be cross-reactivity making interpretation hard especially in animals without proper controls.

Dogs and pigs are probably the only reported domestic animals that have been investigated for infection by ebolaviruses, despite the fact that in Africa, people are more close to livestock than pets or wildlife. Domestic animals especially livestock make good candidates for investigation as a source of infection for humans and secondary reservoirs. This is because they act as an interface between wildlife and humans especially in Africa where some people sleep

in the same house with livestock to avoid thieves and predators. Whereas it is very rare to find pets such as dogs sleeping in the same house with the owner as it is in the Western world, livestock such as goats may share the same accommodation with the owners or in kraals in proximity with the humans. Livestock are handled more by humans through grazing, milking, feeding and all these are done manually. They are taken to the bush in the morning to graze where they interact with wildlife and brought back into the homesteads in the evening.

Our major aim was to investigate whether domestic animals (goats, cattle, sheep, pigs, and dogs) are exposed to ebolaviruses and if they act as potential sources of infection to humans. We present in this paper serological findings from goat samples collected from June-July 2015 post filovirus outbreaks in affected districts from Uganda and compare these with goat samples from DRC and Ivory Coast.

Methods

Study sites and animal blood sample collection in Uganda.

Blood samples were collected from goats from two ecologically different locations in June and July 2015, one in the western Uganda districts of Kamwenge and Ibanda that had been affected by Marburg Virus Disease (MVD) in July 2012 and another in the central district of Luweero that has experienced 2 outbreaks EVD in 2011 and 2012 (Figure 1).

In Western Uganda blood samples were collected from goats owned by households around Kitaka mine in Kasyoho-Kitomi Forest Reserve which is a rich ecosystem bordering Queen Elizabeth National Park. This is also where we find Kitaka mine and Python caves that are inhabited by bat species *Rousettus aegyptiacus* that are reservoirs for Marburg virus (Amman et al., 2012, Amman et al., 2014, Towner et al., 2009).

In central Uganda, Luweero district was chosen purposively because it has reported two outbreaks of *Sudan ebolavirus* in 2011 and 2012 (Albarino et al., 2013, Shoemaker et al., 2012).It is located 60 km north of Uganda's capital city Kampala. The main economic activity in Luweero district is agriculture that involves crop farming and animal husbandry with a grassland savannah type of vegetation. Unlike the western Uganda studied districts of Ibanda and Kamwenge, Luweero district is not known to have bat caves inhabited by bat species *Rousettus aegyptiacus* but has many fruiting trees occupied by different species of bats. Blood samples were collected in collaboration with district veterinary teams of three districts of Ibanda, Kamwnge and Luweero in the months June and July 2015. Districts, sub-counties,

villages were selected purposively. These were the ones considered to be at high risk, either within 50km of caves harboring bats, or have had a previous outbreak of filovirus. Each herd in a household was considered a cluster. Where the herd was more than 15 animals, 25% of the herd was sampled per household, but very few households had more than 15 goats. Goat blood samples were collected in 4ml EDTA vacutainer tubes following standard procedures by veterinarians processed into aliquots and stored at -80°C at Uganda Virus Research Institute (UVRI) Viral Hemorrhagic Fever Laboratory Entebbe Uganda. An animal data collection forms were filled and geographical coordinates collected. Then samples were kept under cold chain for further aliquoting and storage at -80c at UVRI. Additional goat blood samples collected by Robert Koch Institute, Berlin Germany from DRC and Ivory Coast during EVD outbreak investigations were included in the laboratory analysis.

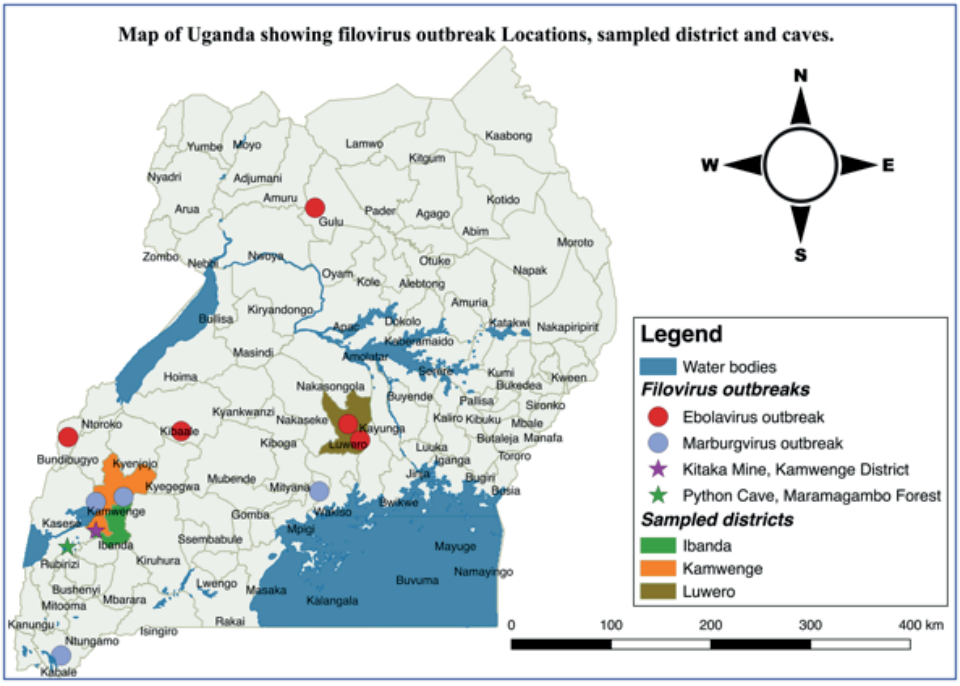


Figure 1. Showing reported filovirus outbreaks in Uganda and sampled districts June-July 2015

Ethical approval.

Approval to conduct this study was obtained from Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) Higher degrees' committee, UVRI Research and Ethics Committee and Uganda National Council of Science and Technology approval number HS 1940. Animal owners were asked to give verbal consent before a blood sample could be collected from their goats and those who did not give consent, their animals were not included.

Veterinary staff involved in the sample collection research were trained and supplied with personal protective equipment (PPE) that they would use during sample collection.

Laboratory Analysis.

Samples were analysed at Robert Koch Institute Berlin Germany, in Research Group 3 Epidemiology of Highly Pathogenic Microorganisms (P3) and Montpellier University, Montpellier, France. Samples were analysed utilising three serological approaches; Enzyme-linked immunosorbent assay (ELISA), Western Blot (WB) analysis and Luminex based assay. Samples were screened first for GP-Zaire reactive IgG antibodies in ELISA, followed by two Western Blot (WB) analyses. WB was utilised to confirm specific reactions against the same antigen. We started with GP only followed by Virus-like particles (VLPs) containing GP, Nucleoprotein (NP) and matrix protein (VP40) and lastly a multiplex luminex based technology assay.

IgG Antibody detection ELISA

ELISA techniques for diagnosing filoviruses have been described before. Some use authentic virus antigens made from virus-infected cells (Johnson et al., 1981, van der Groen et al., 1983, Ksiazek et al., 1999b, Ksiazek et al., 1999a) whereas others use the recombinant-based proteins diagnostic system for filoviruses (Saijo et al., 2001, Nakayama et al., 2010, Prehaud et al., 1998). We used recombinant *Zaire ebolavirus* glycoprotein minus the Transmembrane Region (EBOV rGpdTM) from IBT Bioservices, Inc Rockville USA as the antigen. Positive control was a goat vaccinated with VLPs from Germany and negative control a goat from Germany that had tested negative for ebolaviruses.

Briefly, 96 well ELISA plates (Optical readable micro test plates, sterile from Carl Roth GmbH + Co Karlsruhe, Germany) were coated with 100µl of PBS containing 0.05µg per well of the antigen overnight at 4^0c. The plates were washed three times with PBST using microplate washer (Tecan Trading AG, Switzerland). Then, plates were incubated with 200µl of blocking

buffer solution (5% non-fat dry milk powder in PBS) per well for 1 hour at room temperature at 180rpm. The plate was dried and 100µl samples and negative control added in duplicates in 1:400 blocking buffer dilution as single point measurement. A positive control was added as serial dilution starting with 1:8000.

Samples and controls were incubated on the plate for 2 hours under shaking at 180rpm at room temperature. The plate was washed five times with PBST and incubated for 1 hour with secondary antibody (Horseradish Peroxidase Pure Donkey Anti-goat). Washed six times and incubated with TMB solution under darkness for 10 minutes. The reaction was stopped with 0.25M of Sulphuric acid. Plates were read in ELISA reader (Tecan Trading AG, Switzerland) at OD value 450nm within five minutes after stopping the reaction. Each plate had four blank wells where no sample or controls were added. The OD values of the blanks was subtracted from OD values of wells with samples and controls. The Mean OD value of each duplicate was computed, standard deviation (SD) and precision of measurement. The cut-off for positive samples was generated from Mean OD value of negative control + 3SD, which was 0.2. A sub-set of samples selected from those with very low OD value, intermediate and high OD values from all three countries were taken into Western Blot (WB) testing.

Western Blot Analysis

Only a subset of samples that had high OD values, intermediates, and low was included in Western blot analysis from Uganda, Ivory Coast, and DRC. Western blot has been used to analyze samples for detection of filovirus antibodies (Nakayama et al., 2010). In our case, Western blotting was done in two steps; first samples were tested with GP antigen (EBOV rGpdTM) only followed by virus-like particles (VLPs) expressing recombinant Ebola virus (EBOV) glycoprotein (GP), nucleoprotein (NP), and matrix protein (VP40). These VLPs are produced in sf9 insect cells through infection with recombinant baculovirus procured from IBT Bioservices, Inc Rockville USA. Recombinant GP and VLPs were run on a 10% sodium dodecyl sulfate- polyacrylamide gel under denaturing and reducing conditions. The gel ran for 1 hour at 150V loaded with a colored protein marker XXL DeLuxe(GeneOn GmH Ludwigshafen am Rhein, Germany). The antigens were later transferred from the gel to a polyvinylidene membrane at 15V for 20 minutes. Coated membranes were blocked with blocking buffer (5% non-fat milk powder in PBS) overnight at 4^0c. Membranes were cut into small 3mm strips and placed on western blot plates. Samples were added in 1:100 dilution, negative control in 1:400 dilution, and positive control in 1:4000 dilution all in 1% non-fat

milk powder in PBS, and incubated for 1 hour under shaking at room temperature at 90rpm. Additional controls for the VLPs rabbit anti-EBOV VLPs, anti-VP40 and anti-NP were used. Secondary antibodies (Horseradish peroxidase pure donkey anti-goat and anti-rabbit IgG-HRP) were incubated for 1 hour under shaking at room temperature and detection using TMB solution after incubation for 10 minutes in the dark.

Luminex Assay

The Luminex assay done to test the goat samples has been described previously by the same group that tested these sample (Ayouba et al., 2017). Briefly, this is a serological assay based on Luminex technology for detection of antibodies against Ebolaviruses. It is a sensitive and specific high-throughput serological assay that is important in epidemiological surveys. This multiple analyte profiling technology is a flow cytometry-based system that allows fast and simultaneous detection of up to 100 analytes in a single well of 96-well flat-bottom plate. In this study, we used it to detect antibodies against four of the five species of *Ebolavirus* as has been explained by Ayoub et al., 2017. These include antibodies against *Zaire ebolavirus*, *Sudan ebolavirus*, *Bundibugyo ebolavirus* and *Reston ebolavirus*. A total of ten commercial ebolavirus recombinant antigens were used to assess for antibodies in the goat samples. These include NP for Zaire, GP for Zaire Maying strain, GP for Zaire Kissidougou-Makona strain, VP40 for Zaire, NP for *Sudan ebolavirus*, GP for *Sudan ebolavirus*, VP40 for *Sudan ebolavirus*, GP for *Bundibugyo ebolavirus,* VP40 for *Bundibugyo ebolavirus* and GP for *Reston ebolavirus*

Results

A total of 339 goat blood samples were analyzed with 59.2 % (236/399) from Uganda, 27.1 % (108/399) from Ivory Coast, and 13.8 %(55/399) from DRC. Most of the sampled goats were adults (80.2%), females (81.1%), local indigenous breed (93.6%), and they were healthy at the time of sampling (94.5%).

Ebola virus IgG antibody Seropositivity

From ELISA results, 70 % (282/399) of tested samples had mean OD value above 0.2 which was our cut off for negative samples. We used this cut off conservatively because our positive and negative controls were all goats from Germany hence they had a different ecosystem that could influence their biology and the positive control goat was vaccinated with VLPs. The

mean OD values from Uganda are higher than those of Ivory Coast and DRC (P-value=0.0001) as can be seen in Figure 2.

Figure 3: Plot of the positive control vs negative control used in assessing ELISA results.

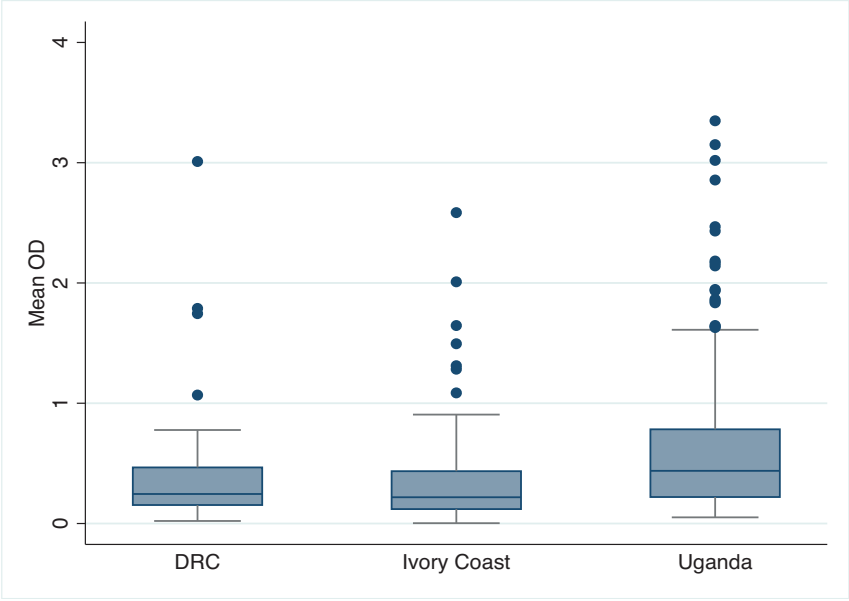
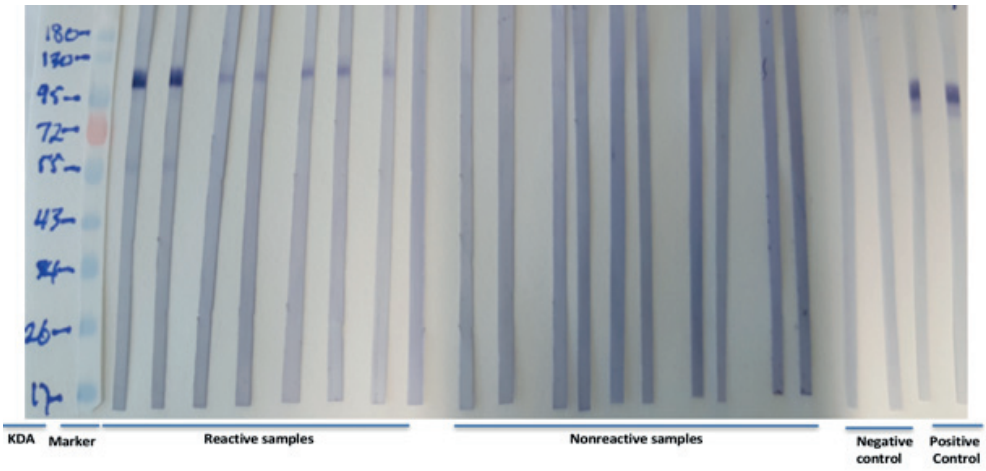


Figure 2: Box plot comparing mean OD values from the three countries.

Only 72 samples were tested in Western blot for GP and VLP, From Table 1, we see that there is some consistency between ELISA results and GP only Western Blot results, but not so much with the VLPs Western Blots. We see that most samples with low OD values were negative on GP only WB and started seeing positives at OD value of 0.55. However this was different with the VLPs which contained three proteins, NP is known to be very sensitive hence even samples that had very low ODs were showing some reactivity on NP compared to GP whereas few samples were reactive with VP40. Given these results, we shifted our ELISA cut off to 0.55 from 0.2 which gave us a seroprevalence of 31.3 % (125/399). For those samples that were run on WB 50 % (36/72) would be considered positive with a cut off OD value of 0.55 and above, but also positive on any of the two WBs. GP only WB, 37.5 % (27/72) would be considered positive, GP in VLPs WB-69.4 % (50/72), NP in VLPs WB-72.2% (52/72) and VP40 in VLPs-6.9 % (5/72). We see that NP and GP antigens embedded within the VLPs are more sensitive

than VP40 and the GP only antigen Western Blots. Figure 3 shows the bands representing the reactivities of some of GP only and VLPs reaction on the membranes in a Westen Blot analysis.

A



B

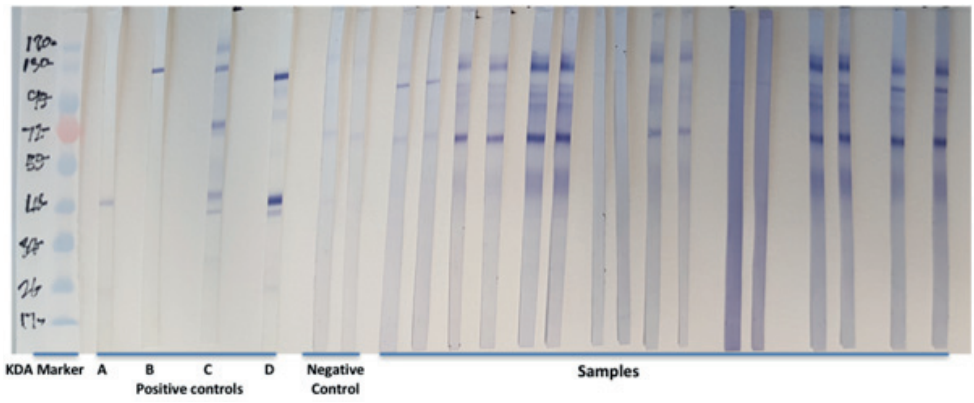


Figure 3: Bands generated against GP(**A**) and VLP(**B**)

Table 1: Comparing ELISA and Western Blot (WB) results

Sample ID	ELISA OD values	WB Reactivity GP only	WB Reactivity VLP_GP	WB Reactivity VLP_NP	WB Reactivity VLP_ VP40	Country	Age	Sex
278G	3.35	++	+	+	(+)	Uganda	Adult	Female
478G	3.15	+	++	++	(+)	Uganda	Adult	Female
784G	3.02	(+)	+	+	(+)	Uganda	Adult	Female
ZN099	3.01	++	+++	+++	+++	DRC	Adult	Female
323G	2.86	(+)	+++	++	++	Uganda	Adult	Female
GPAU282	2.58	+	-	+	-	Ivory Coast	Adult	Female
276G	2.47	++	+++	+++	+++	Uganda	Adult	Female
848G	2.43	++	++	++	-	Uganda	Adult	Male
184G	2.18	++	++	++	-	Uganda	Adult	Female
724G	2.17	++	++	+	(+)	Uganda	Adult	Female
87G	2.14	(-)	++	+	(+)	Uganda	Adult	Female
GPAU329	2.01	+	++	++	-	Ivory Coast	Adult	
386G	1.95	(-)	++	++	(+)	Uganda	Adult	Female
874G	1.94	++	++	+	(+)	Uganda	Adult	Male
95G	1.93	+	++	+	-	Uganda	Adult	Female
84G	1.86	++	++	++	(+)	Uganda	Juvenile	Female
764G	1.85	+++	+	++	-	Uganda	Adult	Female
ZI177	1.79	++	+	+	+	DRC	Juvenile	Male
ZN184	1.74	++	-	(+)	-	DRC	Juvenile	Male
GGAH360	1.65	++	++	+	-	Ivory Coast	Adult	Female
GGOU209	1.49	++	(-)	++	(-)	Ivory Coast	Adult	Female
GGOU211	1.31	++	+	+	-	Ivory Coast	Juvenile	Female
233G	1.25	++	++	+	(+)	Uganda	Adult	Female
1067G	1.16	++	+	++	(+)	Uganda	Adult	Female
001G	1.03	+	+	+	(+)	Uganda	Adult	Female
182G	0.92	+	++	(+)	(+)	Uganda	Adult	Female
92G	0.84	+	++	(+)	(+)	Uganda	Adult	Female
82G	0.74	-	++	+	(+)	Uganda	Adult	Female
582G	0.70	+	++	(+)	(+)	Uganda	Adult	Female

300G	0.67	+	+	+	-	Uganda	Adult	Female
008G	0.61	(-)	+	+	-	Uganda	Juvenile	Female
869G	0.60	-	++	++	(+)	Uganda	Adult	Female
872G	0.59	(+)	++	+	(+)	Uganda	Adult	Female
763G	0.57	(+)	+	+	-	Uganda	Adult	Male
847G	0.57	(+)	(-)	+++	(-)	Uganda	Adult	Female
0011G	0.55	+	++	+	(+)	Uganda	Adult	Female
1032G	0.55	+	+	+	-	Uganda	Adult	Female
260G	0.54	(-)	++	+	(+)	Uganda	Adult	Female
86G	0.54	(-)	+++	+++	+++	Uganda	Adult	Female
268G	0.52	(-)	++	++	(+)	Uganda	Adult	Female
37G	0.51	(+)	+	+	-	Uganda	Juvenile	Male
35G	0.37	(-)	+	+	-	Uganda	Adult	Female
0016G	0.35	(-)	++	++	(+)	Uganda	Adult	Female
299G	0.33	-	(-)	++	-	Uganda	Adult	Female
GZAI453	0.31	-	+	+	-	Ivory Coast	Adult	Female
GDAO0163	0.29	(+)	++	(+)	(+)	Ivory Coast	Adult	Female
GGOU260	0.27	-	+	+	-	Ivory Coast	Adult	Female
GKEI495	0.26	-		-	-	Ivory Coast	Adult	Female
ZM204	0.26	(-)	+	-	-	DRC	Juvenile	Male
GGOU274	0.26	-	-	(+)	-	Ivory Coast	Adult	Female
ZM209	0.22	-	++	+	-	DRC	Adult	Male
781G	0.11	-	-	(+)	-	Uganda	Adult	Male
708G	0.11	-	-	-	-	Uganda	Juvenile	Male
658G	0.11	-	+	+	-	Uganda	Juvenile	Female
697G	0.11	-	+	+	-	Uganda	Juvenile	Female
1028G	0.10	-	-	+	-	Uganda	Adult	Female
0029G	0.10	-	+	+	-	Uganda	Adult	Female
538G	0.10	-	-	(+)	-	Uganda	Adult	Female
ZN180	0.09	-	-	(+)	-	DRC	Adult	Female
1009G	0.09	-	-	+	-	Uganda	Juvenile	Male
GDAO198	0.09	-	-	(-)	-	Ivory Coast	Adult	Female
62G	0.09	-	-	(+)	-	Uganda	Adult	Male
700G	0.09	-	-	(+)	(+)	Uganda	Juvenile	Male
ZN129	0.09	-	+	+	-	DRC	Adult	Female
GTAI086	0.09	-	-	(+)	-	Ivory Coast	Adult	Male
417G	0.08	-	-	(+)	-	Uganda	Adult	Female
597G	0.08	-	+	-	-	Uganda	Adult	Female

GDAO124	0.07	-	-	(-)	-	Ivory Coast	Juvenile	Female
038G	0.06	-	(+)	+	-	Uganda	Juvenile	Female
GPON026	0.06	-	-	-	-	Ivory Coast	Juvenile	Male
695G	0.05	-	+	+	-	Uganda	Adult	Female
GDAO138	0.05	-	-	++	-	Ivory Coast	Juvenile	Male

Luminex Results

In the luminex assay, we see that they were a big number of samples that were reactive (Figure 4). There was more reactivity against GP *Sudan ebolavirus* than other antigens. Over all, GP antigens from all the four-species tested in the multiplex assay were more reactive than NP and VP40. Also, we see a lot of cross reactivity between the four *Ebolavirus* species investigated in this study. Because of lack of positive control, it was hard to develop a proper cut-off for the positive or negative control. Different cut-offs were explored and their results are presented in Figure 17B-F.

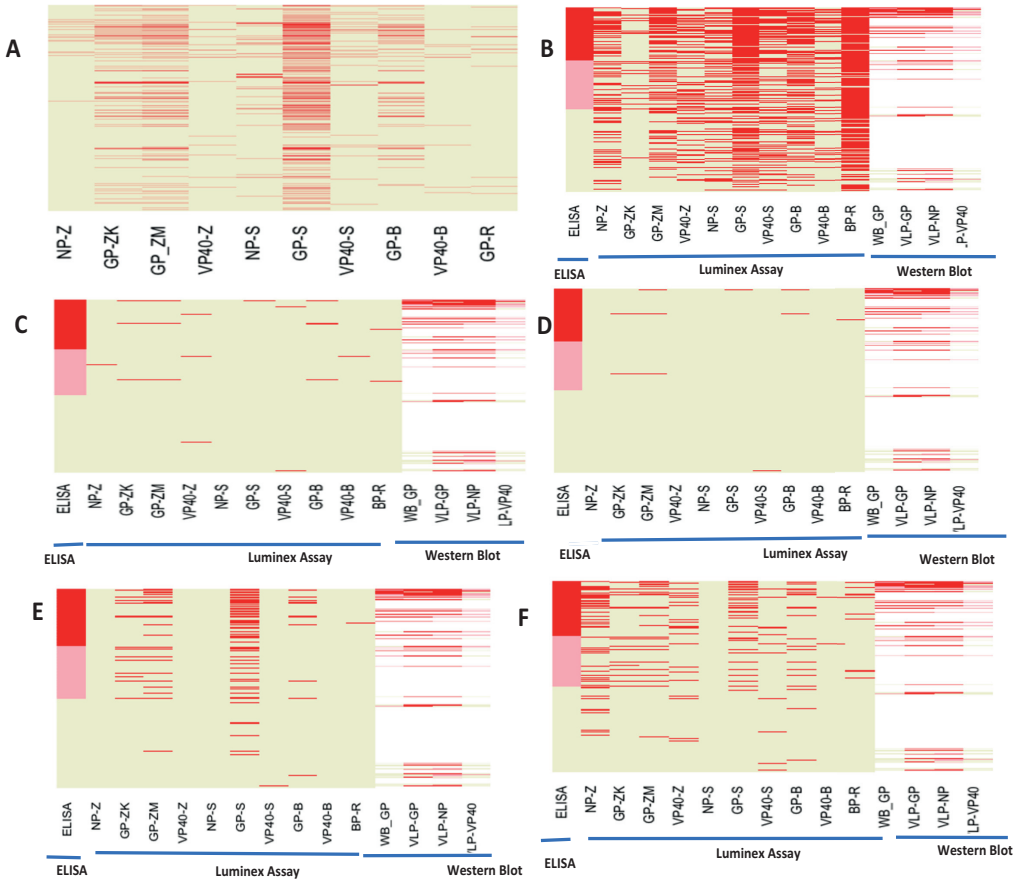


Figure 4: Heat map showing the reactivity of several Ebola virus antigens (GP, NP, VP40) in goat samples from Uganda, DRC, and Ivory Coast (Z=*Zaire ebolavirus*, ZK=*Zaire ebolavirus*, *Kissidougou-Makona strain*, ZM=*Zaire ebolavirus, Mayinga strain*, S=*Sudan ebolavirus*, B=*Bundibugyo ebolavirus*, R=*Reston ebolavirus*, WB=Western Blot, VLP=Virus Like Particles). Red stands for samples with high reactivity or what would be considered positive; pink stands for those samples considered indeterminate, beige color represents samples with low reactivity or those considered negative and white represents samples not tested with that method. **A**: Heat map showing reactivity in Luminex multiplex assay as measured by Median Fluorescence Intensity. **B-F**: compares the results of ELISA and Western Blot to the different cut-offs in the Luminex Multiplex Assay.

Table 2 shows seropositivity following the 0.55 cut off in ELISA of all samples. From Table 2, Uganda had the highest seropositivity at 39.8 % (94/236), Ivory Coast at 21.3 % (23/108) and DRC at 14.6 % (8/55) whereas females and adult goats were more likely to be seropositive given by the statistically significant odds ratios.

Table 2: Bivariate analysis of Seropositivity and goat background characteristics basing on 0.5 ELISA cut-off.

Variable	Number Seropositive (%)	Seronegative (%)	Total	Unadjusted OR(95%CI)	Adjusted OR (95% CI)
Country					
Ivory coast	23(21.3%)	85(78.7%)	108(27.1%)	Ref	
DRC	8(14.6%)	47(85.4%)	55(13.8%)	0.6(0.2 -1.5)	0.7(0.3 -1.8)
Uganda	94(39.8%)	142(60.1%)	236(59.1%)	2.4(1.4-4.1) *	2.4(1.4-4.2) *
Region in Uganda					
Central	37(34.9%)	69(65.1%)	106(44.9%)	ref	
Western	57(42.9%)	73(56.2%)	130(55.1%)	1.5(0.9 - 2.5)	1.2(0.7-2.1)
Sex					
Male	14(18.9%)	60(81.1%)	74(18.9%)	ref	
Female	109(34.3%)	209(65.7%)	318(81.1%)	2.2(1.1 - 4.1) *	2.5(1.04-5.8)
Breed					
Cross	4(26.7%)	11(73.3%)	15(6.4%)	ref	
Local	90(40.2%)	131(59.3%)	221(93.6%)	1.9(0.6 - 6.1)	2.0(0.6-6.9)
Age					
Juvenile	10(12.7%)	69(87.3%)	79(19.8%)	ref	
Adults	115(35.9%)	205(64.1%)	320(80.2%)	3.9(1.9 - 7.8) *	5.3(1.7-16.1)
Production system					
Free range	15(31.9%)	32(68.1%)	47(19.9%)	ref	
Tethering	79(41.8%)	110(58.2%)	189(80.1%)	0.7(0.3 – 1.3)	0.6(0.2-1.1)
Lactating					
No	64(35.9%)	114(64.1%)	178(76.1%)	ref	
Yes	30(53.6%)	26(46.4%)	56(76.1%)	2.1(1.1 – 3.8) *	1.4(0.8-2.7)
Pregnant					
No	56(37.1%)	95(62.9%)	151(64.5%)		
Yes	38(45.8%)	45(54.3)	83(35.5%)	1.4(0.8 -2.5)	0.9(0.5-1.7)
Health Status					
Healthy	80(39.4%)	123(60.6%)	203(86.0%)	ref	
Unhealthy	14(42.4%)	19(57.6%)	33(13.9%)	1.1(0.5- 2.3)	1.1(0.5-2.5)

Discussion

We describe for the first time the detection of IgG antibodies against ebolaviruses in domestic goats (*Capra aegagrus hircus*). A close species, a duiker which belongs to the same family as goats-*Bovidae,* was found to be positive for Ebola virus by RT-PCR (EBOV) (Rouquet et al., 2005). The presence of IgG antibodies against ebolaviruses in goat could be a sign these species could be infected with the filoviruses, and they could be potential reservoirs or at least are potential sources of infection to man.

There were minor differences in seropositivity rate between countries Uganda, DRC and Ivory Coast. Partly due to numbers sampled in these countries, but also the differences in interaction between probable wildlife reservoirs of ebolaviruses and domestic animals. In Uganda for example, there is high human population density, and humans tend to invade the wildlife more compared to DRC and Ivory Coast, and they do so with their animals hence increasing exposure of these domestic animals to filoviruses. Goats, in particular, are browsers and are likely to feed higher in shrubs, tree leaves and fruits which could have been exposed by bats saliva, as research has shown that filoviruses are more likely to be shade orally in saliva than other routes of viral shedding (Amman et al., 2015).

There is a significant difference in seropositivity between male and female goats but not between breed. Also, lactating and pregnant animals had significantly higher antibody responses and seropositivity rates compared to other reproductive status of the animals. We could not find any plausible explanation for this, but this is consistent with what has been found in bats where the infection was high around birthing seasons (Amman et al., 2012). It seems there is a relationship that needs to be investigated between giving birth and infection with filoviruses. Another risk factor for seropositivity was age, where adult goats were four times as likely to be seropositive as juveniles, may be because the older the goats, the higher the chances of being infected and hence seroconversion. Since IgG antibodies can last for as long as 14 years in humans as has been shown for *Sudan ebolavirus* survivors in Uganda (Natesan et al., 2016*)*. This high seropositivity in goats could be due to accumulated cases over time, but this should be interpreted with the life span of domestic goat in question as these goats are not kept for long, because they are usually slaughtered for visitors or sold for income.

The limitation of this study, however, is the reported cross-reactivity of IgG antibodies against filoviruses species. In studies done on filovirus survivors and monkeys vaccinated with VLPs (Kamata et al., 2014, Natesan et al., 2016), it was found that antibodies against *Sudan ebolavirus* would cross-react with those of *Bundibugyo ebolavirus* especially NP. Also in these previous studies, there was cross-reactivity of Marburg virus antibodies with other species of *Ebolavirus*. Although we detect signals generated against recombinant Ebola virus protein GP and VLPs, we cannot be sure that these signals are generated against only Ebola virus. We strongly recommend other diagnostic techniques such as neutralization tests to confirm which filoviruses species are involved or if indeed these reactions seen are against ebolaviruses, not other pathogens or biological mimicry.

We know for example that *Sudan ebolavirus* is circulating in Uganda especially in a Central region where 45% of the Ugandan goats' samples were collected, and Marburg virus circulates especially in Western Uganda where 55% of samples were collected. So some of the signals we are seeing in our results could be due to Marburg virus. This possible cross-reactivity could be seen in a positive sense because it means that infection or exposure by one filovirus species could infer protection against another filovirus. However, this presents a diagnostic challenge.

Because of many species of filovirus that have potential to cross-react with each other especially when the target analyte is IgG, there is need to explore other methods such as Luminex multiplex assay that can detect multiple analytes. In fact, we see cross reactivity across different *Ebolavirus* species from our Luminex assay results, or if indeed these are true antigens against ebolaviruses, then there is a possibility of co-infection with the different *Ebolavirus* species.

Since the majority of the sampled goats were healthy at the time of sampling with no previous reports of sickness, there is a possibility of them having an asymptomatic infection, and RT-PCR would be a helpful technique to use.

Other methods could be cell culture, but these require high biosafety levels and may not be appropriate as for initial animal surveillance hence the need to develop novel methods that could be used in lower biosafety labs especially at the point of care of patients. Indirect fluorescent antibody test that was used to differentiate Ebola virus from a previously isolated Marburg virus requires BSL4 laboratory, which cannot be applied for large-scale animal testing studies. Another antibody detection test that uses viral antigens from inoculated Vero cells as adopted by CDC, which is gamma irradiated and subsequently samples can be run on

lower BSL2 laboratory, but require preparation of the viral antigens and gamma irradiation which is only available in few labs hence also limited for large-scale serological studies. Because of these limitations of viral preparations and gamma irradiations, there has been development of recombinant proteins for use as capture antigens in filovirus testing (Saijo et al., 2006). However, these recombinant proteins have not yet been fully evaluated to date for their sensitivity and specificity, especially in their use in testing animals such as goats. Here we used Recombinant *Ebolavirus* proteins to detect antibodies against Ebola virus in goats in three African countries using both GP and VLPs. The usefulness of these proteins has been evaluated and found to be useful for the use of seroepidemiological studies (Prehaud et al., 1998, Saijo et al., 2006).

Conclusion

We report for the first time the presence of antibodies that react with ebolaviruses GP and VLP antigens in goats. This needs to be investigated further to ascertain if goats are potential reservoirs of ebolaviruses by doing more research such as carrying out neutralisation tests, use of protein AG as the conjuage or get a big negative control dataset from another region.

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APPENDIX II: RESEARCH TOOLS AND ADDITIONAL FILES

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1. S1 Questionnaire. Questionnaire that was used to collect quantitative data. https://doi.org/10.1371/journal.pntd.0005907.s001(PDF)
2. S1 FGD guide. Focused group discussion guide that was used to collect qualitative data. https://doi.org/10.1371/journal.pntd.0005907.s002 (PDF)
3. S1 Fig. This is a picture showing how participants in one of the FGDs ranked the most important clinical signs of Ebola Virus disease.
4. The clinical signs are written in one of the local languages in Uganda, Luganda. https://doi.org/10.1371/journal.pntd.0005907.s003(TIF)
5. S2 Fig. This picture shows proportional piling technique where participants used 100 grains of beans to distribute them according to what they think is most important in transmitting Ebola Virus disease. Words are written in the local language, Luganda. https://doi.org/10.1371/journal.pntd.0005907.s004 (TIF)
6. S3 Fig. The picture shows pairwise ranking technique where participants listed and compared the possible causes of filovirus outbreaks among themselves to come up with a rank of the most important cause. Causes were listed in both rows and columns in the local language. https://doi.org/10.1371/journal.pntd.0005907.s005 (TIF)
7. S4 Fig. The Receiver Operating Curve(ROC) that was used to assess the model for predictors of knowledge towards Ebola and Marburg virus diseases. https://doi.org/10.1371/journal.pntd.0005907.s006 (TIF)
8. S5 Fig. This picture shows proportional piling of 100 grains of beans to determine which gender is affected most by filovirus outbreaks. https://doi.org/10.1371/journal.pntd.0005907.s007 (TIF)
9. S1 Table. Themes and categories generated from focused group discussions by conventional content analysis technique about People's knowledge and attitude towards Ebola and Marburg virus diseases. https://doi.org/10.1371/journal.pntd.0005907.s008 (DOCX)
10. S2 Table. Results of pairwise ranking technique applied on risk factors/causes of Ebola and Marburg virus diseases. https://doi.org/10.1371/journal.pntd.0005907.s009 (DOCX)
11. S3 Table. An alternative model to the logistic regression model if no categorization of knowledge is done.The predictors of knowledge score are the same as those in the logistic regression. https://doi.org/10.1371/journal.pntd.0005907.s010(XLSX)
12. S1 Data. Quantitative data set. https://doi.org/10.1371/journal.pntd.0005907.s011 (XLSX)
13. Occurrence dataset used (Filovirus and Bats Occurrence coordinates)(10.6084/m9.figshare.5306875)
14. Results of the quantitative comparisons of environmental variables to test for multicollinearity(10.6084/m9.figshare.5306908)
15. A jackknife test result to evaluate individual covariate importance in the model developments(10.6084/m9.figshare.5306914)
16. The response curves of all the predictor variables in all the four models(10.6084/m9.figshare.5306932)

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Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no

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