A retrospective cohort investigation of seroprevalence of Marburg virus and ebolaviruses in two different ecological zones in Uganda

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Abstract

Background

Uganda has experienced seven Ebola Virus Disease (EVD) outbreaks and four Marburg Virus disease (MVD) outbreaks between 2000 and 2019. We investigated the seroprevalence and risk factors for Marburg virus and ebolaviruses infections in gold mining communities around Kitaka gold mine in Western Uganda, and compared them to non-mining communities in Central Uganda

Methods

A questionnaire was administered and human blood samples were collected from three exposed groups in Western Uganda (gold miners, and household members of miners, non-miners living within 50 km of Kitaka mine). Controls were community members in Central Uganda far away from any gold mining activity which we considered as low-risk groups or 'unexposed' to filovirus infection. ELISA technique was used to analyse samples, detecting IgG antibodies against Marburg virus and ebolaviruses (filovirus).

Results

Miners in western Uganda were 4.8 times more likely to be seropositive compared to the non-exposed group in central Uganda (RR=4.8, 95%CI 1.3-17.9). Overall, filovirus seropositivity was 2.6% (19/724) of which 2.5% (18/724) was to Sudan virus, 0.1% (1/724) was to Bundibugyo virus, and 0.1% (1/724) to Marburg virus. One individual had IgG antibodies reactive to both Sudan virus and Bundibugyo virus. The risk factors for seropositivity to Sudan virus 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 suspect filovirus cases (3.9, 1.04 -14.5).

Conclusions

These findings indicate that filovirus outbreaks may go undetected in Uganda and people involved in artisan gold mining or living close to mines and/or caves are more likely to be exposed to infection with either Marburg virus or ebolaviruses, likely due to increased risk of exposure to bats. This calls for active surveillance in known high-risk areas for early detection and response to prevent filovirus epidemics.

Background

Viruses in the genera Ebolavirus and Marburgvirus belong to the family Filoviridae and cause classical viral haemorrhagic 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 reported eleven filovirus outbreaks from 2000 to 2019, including seven Ebola virus disease (EVD) outbreaks and four Marburg virus disease (MVD) outbreaks (1). In Ibanda and neighbouring Kamwenge districts of western Uganda, there were two documented outbreaks of MVD (6,7), including one in which cases were gold miners in Kitaka cave (7). Previous studies have found bats of species Rousettus aegyptiacus, sampled

from Kitaka and python caves, to be the known reservoir for Marburg virus (8–10). In 2012, the MVD outbreak in Ibanda district traced the outbreak's origin to villages near Kitaka mines (6). We designed a study to better understand the possible link between artisanal gold mining activities in Kitaka and the transmission of Marburg virus and ebolaviruses in Ibanda and Kamwenge districts. We compared these communities with those in Luweero district where there are no mining operations or cave-inhabited bats, no previously identified human cases of MVD, and there is a different ecological zone.

Methods

Study sites, population, and hypothesis

Study participants were sampled from Ibanda, Kamwenge and Luweero districts (Figure). The bat-inhabited Kitaka mines are located within the boundary of Ibanda and Kamwenge within Kasyoha-Kitomi Forest Reserve. Communities that live in and around this reserve were considered to be at higher risk of exposure to filovirus infection. A comparison group in Luweero district was chosen as a control, "unexposed," group because it is in the Central region of the country far from Kitaka and any other mines, and we hypothesized that Rousettus aegyptiacus bats may not inhabit this region due to lack of suitable habitat, no previously reported MVD cases, and therefore inhabitants would mostly likely be at low risk for exposure to filoviruses.

Sampling procedure, inclusion and exclusion criteria

We sampled three groups of "exposed" individuals in Ibanda and Kamwenge districts that included: 1) Miners and persons that worked in the Kitaka mines from 2007 to 2015, 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 a 50 km radius from any open mining site associated with Kitaka mines, and that were not included in above groups 1 or 2. We then sampled a 4th group of "unexposed" individuals — residents of Luweero district. Members of groups 3 also served as an "unexposed" group when compared to groups 1 and 2. The total sample size was determined to be 724 (291 unexposed and 433 exposed); estimating a 15% seroprevalence of Marburg virus in the exposed groups versus 5% seroprevalence in the unexposed group, with a 95% confidence interval and 80% power. 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 and household members who were willing to participate were included in the study. For groups 3 and 4, a two-stage cluster sampling design was used, with random selection of five sub-counties in each sampling area (sub-counties within 50 km of Kitaka mines in Ibanda/Kamwenge for group 3, entire Luweero district for group 4), followed by random selection of three villages within those sub-districts. In selected villages, the investigators travelled to the location of the main trading post at the village's centre, and participants were chosen following the EPI method (13). Sampling was done in

January and February 2015 in all three districts of Kamwenge, Ibanda and Luweero. Participants that consented to inclusion in the study were interviewed to complete a risk factor questionnaire and provided their answers verbally. One blood sample (4 ml) was collected from each participant for serological testing for filovirus (Marburg virus, Sudan virus, Bundibugyo virus and Ebola virus) IgG by ELISA at Uganda Virus Research Institute (UVRI)/Centres for Disease Control (CDC) VHF laboratory, at Entebbe Uganda.

Data management and Laboratory analysis

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

All the samples collected were tested for the presence of IgG antibodies by Enzyme-Linked Immunosorbent Assay (ELISA), which was validated by US Centres 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% (15).

Briefly, a gamma-irradiated lysate of Vero cells infected with either Sudan virus, Bundibugyo virus, Ebola virus and Marburg virus was used as positive antigen whereas the negative or control antigen had uninfected Vero cells. 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 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 colourless liquid to an intense green colour with a maximum light absorption at 410 nm. The amount of colour developed is proportional to the amount of IgG antibodies which has bound to the antigen on the solid phase. 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 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.

Results

Overall, we sampled 724 individuals, 433 (59.8%) from the high-risk exposure region in Western Uganda (Ibanda and Kamwenge districts) and 291 (40.2%) from the low-risk exposure region in Central Uganda, Luweero district (Table 1). The mean age was 36.3 years (SD = 14.8, 95% CI = 35.2–37.4), the median age was 33.0 years (range 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. Most (67.7%) were farmers, followed by miners at 22.2%.

Table 1

Summary of study groups and corresponding seroprevalences and risk ratios.

Study Cohorts	Number sampled	Marburg virus seroprevalence (%)	Sudan virus (SUDV) seroprevalence (%)	SUDV Risk Ratio (95%CI)
Low-Risk group (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/household member of miner	138	0	4 (2.9%)	2.8(0.64–12.4)
Non-miners within 50 km of Kitaka [b] mine	134	0	3 (2.2%)	2.2(0.44–10.6)
[a] All other groups were compared to the unexposed group as control				
[b] People who live within 50 km of Kitaka cave were not significantly different from miners or their family members.				
*Statistically significant				

In total, 2.6% (19/724) individuals tested had IgG antibodies against filoviruses. Eighteen individuals had Sudan ebolavirus IgG antibodies present (2.5%, 18/724), and one person had IgG antibodies 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. Miners who go inside the caves were 4.8 times more likely to be seropositive for Sudan virus compared to the unexposed group in central Uganda 4.8 (1.3–17.9). Miners and their family members were at higher risk of infection with Sudan virus than the control group from Luweero district (aOR = 3.9, 95% CI 1.1–13.7) (Table 1).

Other risk factors for filovirus seropositivity 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 2

Risk factors for filovirus seropositivity

Variable	Category	Filovirus IgG Seropositive (%)	Filovirus IgG Seronegative (%)	[a]Adjusted OR(95%CI)
Total participants (n = 724)		19(2.6%)	705(97.4%)	
Age (years)	< 20	1(0.96%)	103(99.04%)	
	> 20	18(2.9%)	602(85.4%)	1.9 (0.2–14.7)
Gender	Female	4(1.2%)	329(98.8%)	
	Male	15(3.8%)	376(96.2%)	3.1 (1.01–9.5) *
Education	Never	5(4.1%)	117(95.9%)	
	Primary	10(2.5%)	387(97.5%)	0.4 (0.1–1.3)
	Secondary	4(2.2%)	182(97.9%)	0.3 (0.1–1.4)
	Tertiary	0(0%)	19(100%)	
District	Luweero	3(1.1%)	288(98.9%)	
	Ibanda	9(3.7%)	235(96.3%)	2.4 (0.6–10.2)
	Kamwenge	7(3.7%)	182(96.3%)	2.4 (0.6–10.7)
Famer	No	5(2.2%)	222(97.8%)	
	Yes	14(2.9%)	461(97.5%)	1.3 (0.4–3.6)
Go inside mines	No	10(1.7%)	576(98.3%)	
	Yes	9(6.5%)	129(93.5%)	3.1 (1.2–8.2) *
Contact with bats in mines	No	4(3.5%)	111(96.5%)	
	Yes	5(8.2%)	56(91.8%)	1.9 (0.5–7.4)
Own Domestic animals	No	3(1.8%)	159(98.2%)	

* statistically significant

[a] Adjusted for gender, age and education level

Figure: Reported filovirus outbreaks and studied districts

Variable	Category	Filovirus IgG Seropositive (%)	Filovirus IgG Seronegative (%)	[a]Adjusted OR(95%CI)
	Yes	16(2.8%)	546(97.2%)	1.3 (0.4–4.8)
Contact with Animals	No	2(1.2%)	161(22.8%)	
	Yes	17(3.1%)	544(96.9%)	3.7 (0.4–36.3)
Hunting	No	14(2.3%)	582(97.7%)	
	Yes	5(3.9%)	123(96.1%)	1.1 (0.4–3.4)
Contact with dead animals	No	16(2.5%)	621(97.5%)	
	Yes	3(4.5%)	63(95.5%)	1.4 (0.4–4.9)
Eat bush meat	No	6(1.6%)	371(98.4%)	
	Yes	13(3.7%)	334(96.3%)	2.0 (0.7–5.6)
Cleaning of dead body	No	12(2%)	590(98.0%)	
	Yes	5(5.8%)	81(94.2%)	3.1 (1.04–9.1)*
MVD reported in the village	No	11(1.9%)	563(98.1)	
	Yes	8(5.3%)	142(94.7%)	2.2 (0.8–6.2)
Contact with EVD/MVD suspects	No	16(2.3%)	677(97.7%)	
	Yes	3(9.7%)	28(90.3%)	3.9 (1.04–14.5)*
Frequently travels	No	7(5.9%)	112(94.1%)	
	Yes	12(2%)	593(98.0%)	0.3(0.1–0.7)*
Go to the Forest frequently	No	4(1.6%)	240(98.4%)	
	Yes	15(3.1%)	465(96.9%)	2.(0.7–6.1)

* statistically significant

[a] Adjusted for gender, age and education level

Figure: Reported filovirus outbreaks and studied districts

Variable	Category	Filovirus IgG Seropositive (%)	Filovirus IgG Seronegative (%)	[a]Adjusted OR(95%CI)
Wash fruits before eating	No	13(3.0)	417(96.9%)	
	Yes	6(2.2%)	264(97.8%)	0.9(0.3–2.4)
Reported of bats in the house	No	4(1.3%)	313(98.7%)	
	Yes	15(3.7%)	392(96.3%)	2.5(0.8–7.8)
* statistically significant				
[a] Adjusted for gender, age and education level				
Figure: Reported filovirus outbreaks and studied districts				

Discussion

Comparing the two groups of people, one living near an ecosystem of bat in habited caves and forest reserves in western Uganda and another in savanna rangeland in central Uganda, we see that the risk of being filovirus positive is higher in the former group than the later by at least four times. Geographical differences that favour 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 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 filovirus seroprevalence in our high-risk exposure group in Ibanda and Kamwenge district in Western Uganda at 3.7% (16/433) compared to low-risk exposure group in Central Uganda at 1.1% (3/291). Artisanal gold miners who enter bat-inhabited caves had an even higher prevalence of 5.6% (9/161) compared to non-miners in central Uganda at 1% (3/291) As has been reported before, the Kitaka mines where the exposed population is centred is inhabited by bats of species Rousettus aegyptiacus that are the known reservoirs for Marburg virus (7–10). We expected a higher seroprevalence against Marburg virus than Ebola virus, but the opposite was observed with Sudan virus seroprevalence 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 (6, 7), 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. Another possibility for this finding is there could 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 contrasts with our low-risk exposure group 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 high-risk exposure region, and he did not show seroprevalence for any other filovirus species.

Our findings are also consistent with 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 (21, 24, 34). 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. It is important to note the miners in Kitaka cave live in forested ecosystem near a national park that is comparable to the central African forest.

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%) (45). This further indicates that communities that live in the forested areas, like the ones we studied in western 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 fruit 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 (46) 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 Uganda (AOR = 3.4, 1.3–8.5). In fact, the very first cases of Ebola virus were reported in mining communities in DRC in 1976. Other factors that we identified as risk factors were being 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 seropositive individuals we found in the Luweero district 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 virus seropositivity has before been reported in a grassland savanna-like ecosystem in Nigeria similar to the grassland savannah ecosystem of Luweero where the four EVD (4) seropositive cases were identified (36). However, frequent travels outside high-risk areas were protective (0.3; 0.1–0.7). This may be because those who frequently travel away from risk areas are less likely to be 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, likely partly due to men being more likely to be miners and go inside the mines and the forests for manual work and become exposed and hence acquire infection. Cleaning a dead body was significantly 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 a possible higher level of infection control practiced in hospitals in Uganda. Contact with EVD/MVD suspect was a

predictor of seropositivity with a filovirus and has been reported in a partial meta-analysis done on the risk of Ebola transmissions (47).

Looking at the overall seroprevalence reported in this study, the findings suggest that there may be filovirus infections that 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 are at higher risk of infection with filoviruses, likely because of their increased risk of exposure to bats that inhabit those mines and caves. This could possibly lead to large epidemics as was seen in West Africa (16). Additionally, our findings do not rule out the possibility that there could be cross-reactivity for filoviruses in our diagnostic assays caused by either another filovirus infection or a non-filovirus infection.

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 Bundibugyo virus species, as has been described previously. However, a similar unpublished study was carried out by CDC and the Ministry of Health following 2007 MVD outbreak in Kamwenge and Ibanda district in the same area. In that study, they found seroprevalence of Marburg virus at 1.2% (7/564) and Sudan virus at 1.2% (7/564). The seroprevalence of Marburg virus was slightly lower in our study whereas that of ebolaviruses was higher. We do not have a clear explanation for these differences in seropositivity between the two studies conducted in the same area eight years apart. Also, the seroprevalence in our study is lower than 3% and 8% for pooled seroprevalences reported in meta-analyses of seroprevalence of ebolaviruses performed in other parts of the world (1, 17). Following the West Africa EVD outbreak, reports of asymptomatic infection in West African populations has been suggested in populations who had contact with EVD patients at 12%, and at 2.6% in non-contacts (18, 19).

Our study also reported a lower seroprevalence of Ebolaviruses (2.5%) than pooled seroprevalences reported in other studies in neighbouring Democratic Republic of Congo (DRC) at 10% (20–25), Central African Republic and Gabon at 11% (26–30), Sudan at 22% (31), Madagascar at 4% (32), Liberia at 13% (33) and Cameroon at 7% (34, 35), However, our study showed higher seroprevalence than that reported in Nigeria at 2% (36), Germany at 1% (37) and Kenya at 1% (38). Only one Marburg virus seropositive person was confirmed in our study and this was much lower than has been reported in other studies (32, 37–42).

We also report for the first time seroprevalence of Bundibugyo ebolavirus in one individual, while no individuals showed seroprevalence for Ebola Zaire virus. These variations in seroprevalence could be due to differences in filovirus ELISA testing protocols and potential cross-reactivity caused by a non-filoviral infection. 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 (15). 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 (43, 44). 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 can potentially overestimate the true level of seropositivity and therefore overestimate risk and exposure due to varying cross reactivity between differing and unvalidated serological assays used in previous studies. We are continuing to classify these serological results with validation assays including viral neutralization in order to confirm if our findings represent true undetected filovirus infections in these communities, or through cross-reaction with other viral infections, or variability in serologic assays performed.

Conclusions

We conclude that filovirus infections may go undetected by the health care system in Uganda. Also, miners in Ibanda and Kamwenge, near Kitaka mine, are at higher risk of filovirus infection compared to populations living further away from these cave environments. Increased surveillance is still critical in detecting and quickly averting future widespread and devastating filovirus epidemics.

Abbreviations

EVD
Ebola Virus Disease; MVD:Marburg Virus Disease, ELISA:Enyme-linked
IgG
Immunoglobulin G
AOR
Adjusted Odds Ratio
VHF
Viral Haemorrhagic Fever
EPI
Expanded Program for Immunization
UVRI
Uganda Virus Research Institute
CDC
United States Centres for Disease Control and Prevention
OR
Odds Ratio
PBS
Phosphate buffered Saline.
Ag
Antigen

ABTS
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
OD
Optical Density
NTF
National Task Force
SD
Standard Deviation
RR
Risk Ratio
DRC
Democratic Republic Congo

Declarations

Ethics Approval and consent to participate

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.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

Authors declare no competing interest in this paper

Authors' contributions

Conceived and designed the protocol: LN, TS, SB, IS SN, PR. Data collection and Analysis: LN, AT, SB JK, SM. Manuscript preparation: LN, IS, TS, SB, SM, AT, JK, BK, JL,PR, SN, . All authors read and approved the final manuscript.

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Disclaimer

“The opinions expressed by authors contributing to this article do not necessarily reflect the opinions of the Centers for Disease Control and Prevention or the institutions with which the authors are affiliated.”

Biographical Sketch

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Nyakarahuka L, Kankya C, Krontveit R, Mayer B, Mwiine FN, Lutwama J, et al. 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. 2016 Nov 25;16(1):708.
2. Oyok T, Odonga C, Mulwani E, Abur J, Kaducu F, Akech M, et al. Outbreak of Ebola hemorrhagic fever - Uganda, August 2000-January 2001 (Reprinted from MMWR, vol 50, 73-77, 2000). Jama-J Am Med Assoc. 2001 Feb 28;285(8):1010-2.

3. Shoemaker T, MacNeil A, Balinandi S, Campbell S, Wamala JF, McMullan LK, et al. Reemerging Sudan Ebola virus disease in Uganda, 2011. Emerg Infect Dis. 2012 Sep;18(9):1480-3.
4. Mbonye A, Wamala J, Winyi K, Tugumizemo V, Aceng J, Makumbi I. Repeated outbreaks of viral hemorrhagic fevers in Uganda. Afr Health Sci. 2012 Dec;12(4):579-83.
5. Albarino CG, Shoemaker T, Khristova ML, Wamala JF, Muyembe JJ, Balinandi S, et al. Genomic analysis of filoviruses associated with four viral hemorrhagic fever outbreaks in Uganda and the Democratic Republic of the Congo in 2012. Virology. 2013 Aug 1;442(2):97-100.
6. Knust B, Schafer IJ, Wamala J, Nyakarahuka L, Okot C, Shoemaker T, et al. Multidistrict Outbreak of Marburg Virus Disease-Uganda, 2012. J Infect Dis. 2015 Oct 1;212 Suppl 2:S119-28.
7. Adjemian J, Farnon EC, Tschioko F, Wamala JF, Byaruhanga E, Bwire GS, et al. Outbreak of Marburg hemorrhagic fever among miners in Kamwenge and Ibanda Districts, Uganda, 2007. J Infect Dis. 2011 Nov;204 Suppl 3:S796-9.
8. Amman BR, Carroll SA, Reed ZD, Sealy TK, Balinandi S, Swanepoel R, et al. Seasonal pulses of Marburg virus circulation in juvenile Rousettus aegyptiacus bats coincide with periods of increased risk of human infection. PLoS Pathog. 2012;8(10):e1002877.
9. Amman BR, Nyakarahuka L, McElroy AK, Dodd KA, Sealy TK, Schuh AJ, et al. Marburgvirus resurgence in Kitaka Mine bat population after extermination attempts, Uganda. Emerg Infect Dis. 2014 Oct;20(10):1761-4.
10. Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog. 2009 Jul;5(7):e1000536.
11. Centers for Disease C, Prevention. Imported case of Marburg hemorrhagic fever - Colorado, 2008. MMWR Morb Mortal Wkly Rep. 2009 Dec 18;58(49):1377-81.
12. Timen A, Koopmans MP, Vossen AC, van Doornum GJ, Gunther S, van den Berkmortel F, et al. Response to imported case of Marburg hemorrhagic fever, the Netherland. Emerg Infect Dis. 2009 Aug;15(8):1171-5.
13. Bennett S, Woods T, Liyanage WM, Smith DL. A simplified general method for cluster-sample surveys of health in developing countries. World Health Stat Q. 1991;44(3):98-106.
14. Viera AJ. Odds ratios and risk ratios: what's the difference and why does it matter? Southern medical journal. 2008;101(7):730-4.
15. Ksiazek TG, West CP, Rollin PE, Jahrling PB, Peters CJ. ELISA for the Detection of Antibodies to Ebola Viruses. J Infect Dis. 1999 February 1, 1999;179(Supplement 1):S192-S8.
16. Ebola virus disease in West Africa--the first 9 months of the epidemic and forward projections. N Engl J Med. 2014 Oct 16;371(16):1481-95.
17. Bower H, Glynn JR. A systematic review and meta-analysis of seroprevalence surveys of ebolavirus infection. Sci Data. 2017;4.
18. Glynn JR, Bower H, Johnson S, Houlihan CF, Montesano C, Scott JT, et al. Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-

sectional study using a new non-invasive assay for antibodies to Ebola virus. Lancet Infect Dis. 2017.

19. Richardson ET, Kelly JD, Barrie MB, Mesman AW, Karku S, Quiwa K, et al. Minimally Symptomatic Infection in an Ebola 'Hotspot': A Cross-Sectional Serosurvey. PLoS Negl Trop Dis. 2016;10(11):e0005087.
20. Busico KM, Marshall KL, Ksiazek TG, Roels TH, Fleerackers Y, Feldmann H, et al. Prevalence of IgG antibodies to Ebola virus in individuals during an Ebola outbreak, Democratic Republic of the Congo, 1995. J Infect Dis. 1999 Feb;179 Suppl 1:S102-7.
21. Becquart P, Wauquier N, Mahlakõiv T, Nkoghe D, Padilla C, Souris M, et al. High prevalence of both humoral and cellular immunity to Zaire ebolavirus among rural populations in Gabon. PLoS One. 2010;5(2):e9126.
22. Heymann D, Weisfeld J, Webb P, Johnson K, Cairns T, Berquist H. Ebola hemorrhagic fever: Tandala, Zaire, 1977–1978. J Infect Dis. 1980;142(3):372-6.
23. Commission I. Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ. 1978;56(2):271-93.
24. Nkoghe D, Padilla C, Becquart P, Wauquier N, Moussavou G, Akué JP, et al. Risk factors for Zaire ebolavirus–specific IgG in rural Gabonese populations. J Infect Dis. 2011;204(suppl 3):S768-S75.
25. Van der Groen G, Pattyn S. Measurement of antibodies to Ebola virus in human sera from NW-Zaire. Ann Soc Belg Med Trop. 1979;59(1):87-92.
26. Allela L, Boury O, Pouillot R, Delicat A, Yaba P, Kumulungui B, et al. Ebola virus antibody prevalence in dogs and human risk. Emerg Infect Dis. 2005 Mar;11(3):385-90.
27. Heffernan RT, Pambo B, Hatchett RJ, Leman PA, Swanepoel R, Ryder RW. Low seroprevalence of IgG antibodies to Ebola virus in an epidemic zone: Ogooue-Ivindo region, Northeastern Gabon, 1997. J Infect Dis. 2005 Mar 15;191(6):964-8.
28. Lahm SA, Kombila M, Swanepoel R, Barnes RF. Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994–2003. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2007;101(1):64-78.
29. Georges A-J, Leroy EM, Renaut AA, Benissan CT, Nabias RJ, Ngoc MT, et al. Ebola hemorrhagic fever outbreaks in Gabon, 1994–1997: epidemiologic and health control issues. J Infect Dis. 1999;179(Supplement 1):S65-S75.
30. Bertherat E, Renaut A, Nabias R, Dubreuil G, Georges-Courbot MC. Leptospirosis and Ebola virus infection in five gold-panning villages in northeastern Gabon. Am J Trop Med Hyg. 1999 Apr;60(4):610-5.
31. Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. Bull World Health Organ. 1983;61(6):997.
32. Mathiot CC, Fontenille D, Georges AJ, Coulanges P. Antibodies to haemorrhagic fever viruses in Madagascar populations. Trans R Soc Trop Med Hyg. 1989 May-Jun;83(3):407-9.

33. Meunier D, Johnson E, Gonzalez J, Georges-Courbot M, Madelon M, Georges A. Current serologic data on viral hemorrhagic fevers in the Central African Republic. Bull Soc Pathol Exot Filiales. 1986;80(1):51-61.
34. Bouree P, Bergmann J-f. Ebola virus infection in man: a serological and epidemiological survey in the Cameroons. Am J Trop Med Hyg. 1983;32(6):1465-6.
35. Paix MA, Poveda JD, Malvy D, Bailly C, Merlin M, Fleury HJ. [Serological study of the virus responsible for hemorrhagic fever in an urban population of Cameroon]. Bull Soc Pathol Exot Filiales. 1988;81(4):679-82.
36. Tomori O, Fabiyi A, Sorungbe A, Smith A, McCormick JB. Viral hemorrhagic fever antibodies in Nigerian populations. Am J Trop Med Hyg. 1988 Mar;38(2):407-10.
37. Becker S, Feldmann H, Will C, Slenczka W. Evidence for occurrence of filovirus antibodies in humans and imported monkeys: do subclinical filovirus infections occur worldwide? Med Microbiol Immunol. 1992;181(1):43-55.
38. Johnson BK, Ocheng D, Gichogo A, Okiro M, Libondo D, Tukei PM, et al. Antibodies against haemorrhagic fever viruses in Kenya populations. Trans R Soc Trop Med Hyg. 1983;77(5):731-3.
39. Van der Waals FW, Pomeroy KL, Goudsmit J, Asher DM, Gajdusek DC. Hemorrhagic fever virus infections in an isolated rainforest area of central Liberia. Limitations of the indirect immunofluorescence slide test for antibody screening in Africa. Trop Geogr Med. 1986 Sep;38(3):209-14.
40. Gonzalez JP, Josse R, Johnson ED, Merlin M, Georges AJ, Abandja J, et al. Antibody prevalence against haemorrhagic fever viruses in randomized representative Central African populations. Res Virol. 1989 Jul-Aug;140(4):319-31.
41. Johnson E, Gonzalez J-P, Georges A. Filovirus activity among selected ethnic groups inhabiting the tropical forest of equatorial Africa. Trans R Soc Trop Med Hyg. 1993;87(5):536-8.
42. Johnson E, Gonzalez J-P, Georges A. Haemorrhagic fever virus activity in equatorial Africa: distribution and prevalence of filovirus reactive antibody in the Central African Republic. Trans R Soc Trop Med Hyg. 1993;87(5):530-5.
43. Macneil A, Reed Z, Rollin PE. Serologic cross-reactivity of human IgM and IgG antibodies to five species of Ebola virus. PLoS Negl Trop Dis. 2011 Jun;5(6):e1175.
44. Natesan M, Jensen SM, Keasey SL, Kamata T, Kuehne AI, Stonier SW, et al. Human Survivors of Disease Outbreaks Caused by Ebola or Marburg Virus Exhibit Cross-Reactive and Long-Lived Antibody Responses. Clin Vaccine Immunol. 2016 Aug;23(8):717-24.
45. Gonzalez JP, Nakoune E, Slenczka W, Vidal P, Morvan JM. Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic. Microbes Infect. 2000 Jan;2(1):39-44.
46. Bausch DG, Borchert M, Grein T, Roth C, Swanepoel R, Libande ML, et al. Risk factors for Marburg hemorrhagic fever, Democratic Republic of the Congo. Emerging infectious diseases. 2003;9(12):1531-7.

47. Brainard J, Hooper L, Pond K, Edmunds K, Hunter PR. Risk factors for transmission of Ebola or Marburg virus disease: a systematic review and meta-analysis. International journal of epidemiology. 2015:dyv307.

Figures

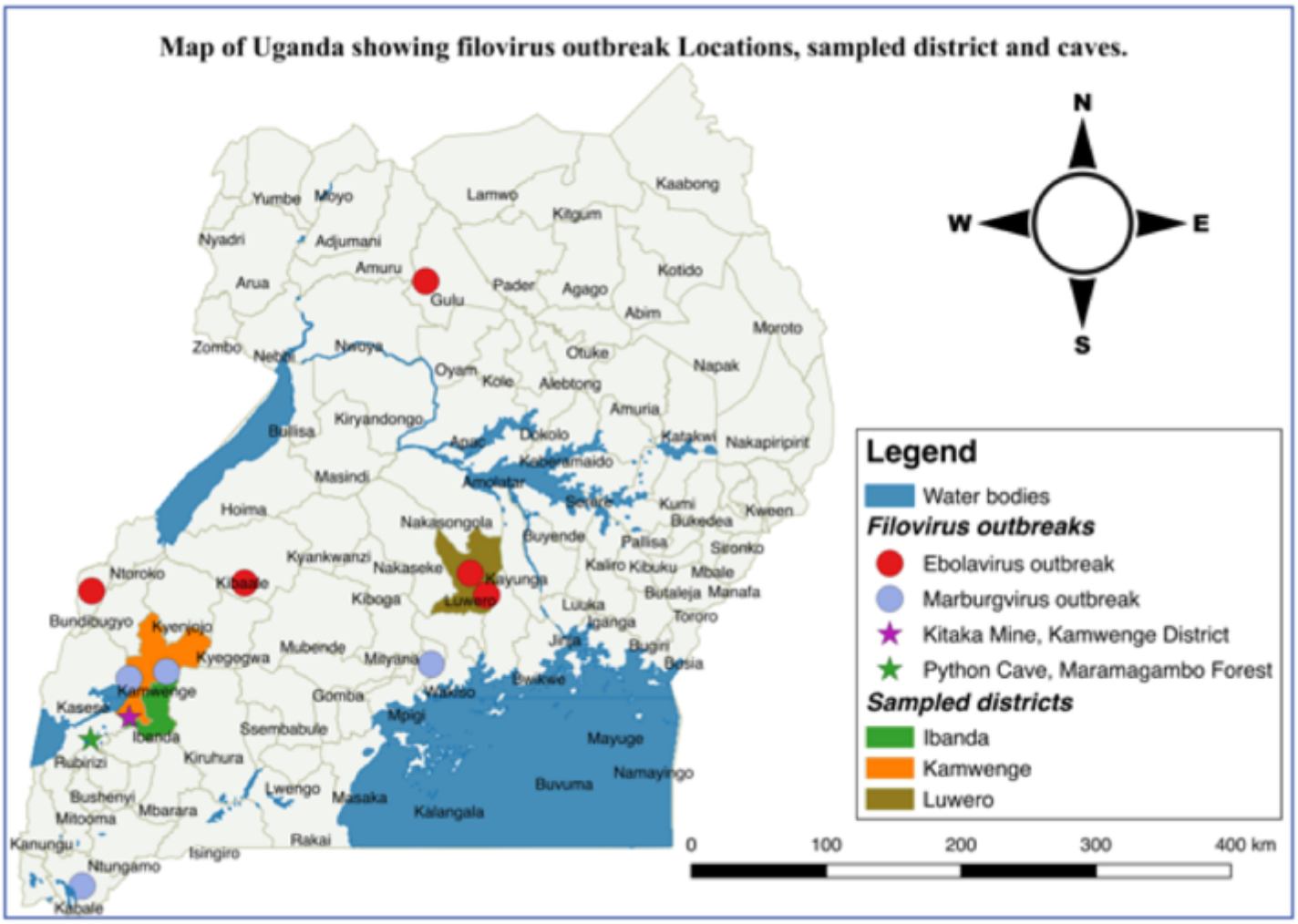

Figure 1

Reported filovirus outbreaks and studied districts