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SUPPLEMENT ARTICLE

Suspected Exposure to Filoviruses Among People Contacting Wildlife in Southwestern Uganda

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Background. Human and filovirus host interactions remain poorly understood in areas where Ebola hemorrhagic fever outbreaks are likely to occur. In the Bwindi region of Uganda, a hot spot of mammalian biodiversity in Africa, human livelihoods are intimately connected with wildlife, creating potential for exposure to filoviruses.

Methods. We tested samples from 331 febrile patients presenting to healthcare facilities near Bwindi Impenetrable Forest, Uganda, by polymerase chain reaction (PCR) analysis and Western blot, using recombinant glycoprotein antigens for Ebola virus (EBOV), Sudan virus (SUDV), Bundibugyo virus (BDBV), and Marburg virus. Behavioral data on contact with wildlife were collected to examine risk factors for filovirus seropositivity.

Results. All patients were negative for active filovirus infection, by PCR analysis. However, patients were seroreactive to SUDV (4.7%), EBOV (5.3%), and BDBV (8.9%), indicating previous exposure. Touching duikers was the most significant risk factor associated with EBOV seropositivity, while hunting primates and touching and/or eating cane rats were significant risk factors for SUDV seropositivity.

Conclusions. People in southwestern Uganda have suspected previous exposure to filoviruses, particularly those with a history of wildlife contact. Circulation of filoviruses in wild animals and subsequent spillover into humans could be more common than previously reported.

Keywords. Filovirus; Ebola virus; Marburg virus; zoonoses; bushmeat; wildlife; Uganda.

Since the discovery of filoviruses 5 decades ago, Ebola hemorrhagic fever (EHF) and Marburg hemorrhagic fever (MHF) outbreaks have become more frequently recognized and pose great risk for regional and international spread [1]. Increased contact between humans and wildlife in regions where wildlife hosts live or migrate is likely contributing to this rise in filovirus outbreaks [2]. Elucidation of human and animal host interactions are needed to institute behavioral changes and risk mitigation aimed at prevention in regions where hemorrhagic fever outbreaks are prone to occur.

Based on field and experimental studies, *Pteropodidae* fruit bats have been indicated as reservoirs for Marburg virus (MARV) and suggested as possible hosts for Ebola virus (EBOV) [3–5]. Direct exposure to fruit bats through consumption and close proximity (eg, by sharing dwellings and entering mines or caves) has been linked to human EHF and MHF outbreaks in Uganda and other parts of East/Central Africa [6–9]. In addition, MARV and EBOV sequences have been detected in insectivorous bats (*Rhinolophus eloquens*, *Miniopterus inflatus*, and *Hipposideros* species), but no links to human EHF and MHF have been reported [4, 10]. It is unknown whether filoviruses are circulating regularly in bat populations.

Contact with nonhuman primates has also been implicated as a source of human infections with EBOV and Taï Forest virus (TAFV) [11, 12]. However, nonhuman primate species infected with EBOV and TAFV have had severe or fatal illness, suggesting that they are not suitable reservoir species but could potentially transmit filoviruses to humans when hunted and handled after death [12–14]. Members of the *Ebolavirus* genus have been implicated as one of the causes of the decline of wild common chimpanzees (*Pan troglodytes*) and western lowland gorillas (*Gorilla gorilla gorilla*) [14, 15] and are considered a major threat to the survival of African great apes [16].

Limited evidence exists for other wild and domestic animal species potentially playing a role in filovirus transmission to either humans and/or other great apes. Duikers (*Cephalophus* species), small forest antelopes, have been implicated as incidental hosts for EBOV [13]. An increase in brush-tailed porcupine (*Atherurus africanus*) and wild bush pig (*Potamochoerus larvatus*) mortality has also temporally and geographically coincided with human EHF outbreaks [17]. In addition, EBOV RNA has been detected in 2 Muridae rodent genera and 1 shrew species (*Sylvisorex ollula*), although attempts to isolate the virus were unsuccessful [18]. Swine have been confirmed as susceptible domestic animal hosts for Reston virus [19] and

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immunoglobulin G (IgG) EBOV antibodies have been detected in sera from domestic dogs in close proximity to an EBOV epidemic area [20].

Despite multiple pieces of evidence, further investigation and confirmation of potential wildlife reservoirs and additional incidental hosts for filoviruses is lacking, partly due to minimal systematic wildlife surveillance efforts and the availability of accurate diagnostic tests. Challenges which have hampered filovirus surveillance in wildlife include a very short time frame within which to detect viral shedding in animals involved in initial spillover of filoviruses to humans and difficulties in achieving necessary sample sizes in suspected animal reservoirs, particularly among species with unknown population size. Limitations in availability of accurate diagnostic testing include requirement of maximum containment (biosafety level 4) facilities to conduct virus isolation and cross-reactivity between filovirus species among available serological assays. In addition, surveillance and diagnostic capabilities are likely complicated by the presence of unknown filoviruses, which have the potential to cross-react with serological diagnostics and influence immunological susceptibility of reservoir and incidental hosts.

In consideration of these challenges facing wildlife studies, human serological assays optimized for detection of filoviruses, combined with epidemiologic data on human-wildlife contact, could provide valuable insight into the geographic distribution of filoviruses, as well as the wildlife populations in which to focus further studies. Previous human serological studies have linked activities such as scavenging gorilla, chimpanzee, and duiker carcasses to EHF outbreaks in Gabon [12, 13, 21] and have identified forest-dwelling human populations and people living in close proximity to forests in the Central African Republic and the Democratic Republic of Congo (DRC) as high-risk human groups for EBOV exposure [22, 23].

In this study, we investigated potential exposures to filoviruses among acutely febrile patients from the Bakiga and Batwa tribes in southwestern Uganda, and identified possible risk factors for filovirus seropositive status using detailed livelihood and behavior surveys. The Bakiga are agriculturalists, whereas the Batwa were previously hunter-gatherers prior to their displacement from the interior of the Bwindi Impenetrable Forest. The Batwa now live in settlements surrounding Bwindi Impenetrable National Park and have become one of the most marginalized human groups in Uganda. The park dominates this region and is a biodiversity hotspot, harboring among the largest number of primate species of any forest in Africa, including the critically endangered mountain gorilla (*Gorilla beringei beringei*) [24]. Our study objectives were to (1) identify whether humans in the Bwindi region have had past exposures to filoviruses, by testing for the presence of IgG antibodies to specific filovirus glycoproteins (GPs), and (2) identify wildlife species and types of wildlife interactions associated with human exposure to filoviruses.

METHODS

Human Subjects

A total of 331 patients from Bwindi Community Hospital and Byumba Health Center II presenting with fever and accompanying clinical signs were voluntarily enrolled in the study between March and June 2013 (Figure 1). Inclusion criteria included nonspecific signs of viral illness, such as fever (ie, body temperature >37.8°C), vomiting, diarrhea, abdominal pain, nasal congestion, and/or cough. Blood samples and oropharyngeal swab specimens were collected from each participant. Questionnaires to collect demographic information, travel history, medical history, information on livelihood(s), and interactions with domestic and wild animals were administered in the local language, Runyankore-Rukiga. The aim of the study was communicated to all patients or parents/guardians of minors, and written informed consent was obtained from all study participants. Institutional review boards at Makerere University, Bwindi Community Hospital, the University of California–Davis, and the Uganda National Council for Science and Technology approved this study.

Polymerase Chain Reaction (PCR)–Based Diagnostic Techniques

Total nucleic acid was extracted from whole-blood and oral swab specimens, using the NucliSens MiniMag system (bioMérieux) according to manufacturer's instructions, with the exception of the addition of a 2-hour proteinase K digestion step with mechanical disruption for whole-blood samples. RNA was reverse transcribed and complementary DNA (cDNA) synthesized using Superscript III First-Strand Synthesis cDNA kits (Invitrogen, Carlsbad, CA). cDNA was analyzed by conventional PCR analysis for the filovirus L gene, using primers previously designed by Zhai et al [25]. A touchdown protocol was programmed, using a Fast Cycling PCR kit (Qiagen, Germantown, MD), with the following reaction conditions: 1 cycle at 95°C for 5 minutes; 12 cycles at 96°C for 8 seconds, 65°C (with a 1°C decrease during each subsequent cycle) for 8 seconds, and 68°C for 18 seconds; 35 cycles at 96°C for 8 seconds, 52°C for 8 seconds, and 68°C for 18 seconds; and 1 cycle at 72°C for 5 minutes. PCR products of appropriate size were cloned using Topo TA cloning kits (Invitrogen), and sequencing was performed using Sanger dideoxy sequencing at the University of California–Davis DNA sequencing laboratory.

Western Blots

Samples were γ-irradiated with a dose of 2 MRad before screening [26]. Samples were denatured in 2.5% β-mercaptoethanol, and proteins were separated on 10% mini-protean TGX gels (Biorad, Hercules, CA) and transferred to Amersham Hybond 0.45 polyvinylidene fluoride membranes (GE-biosciences, Marlborough, MA). Blots were blocked in 5% nonfat milk and 1% normal goat serum. Membranes were incubated with serum at a 1:100 dilution overnight at room temperature. Membranes were then incubated with peroxidase-labeled antibody to human

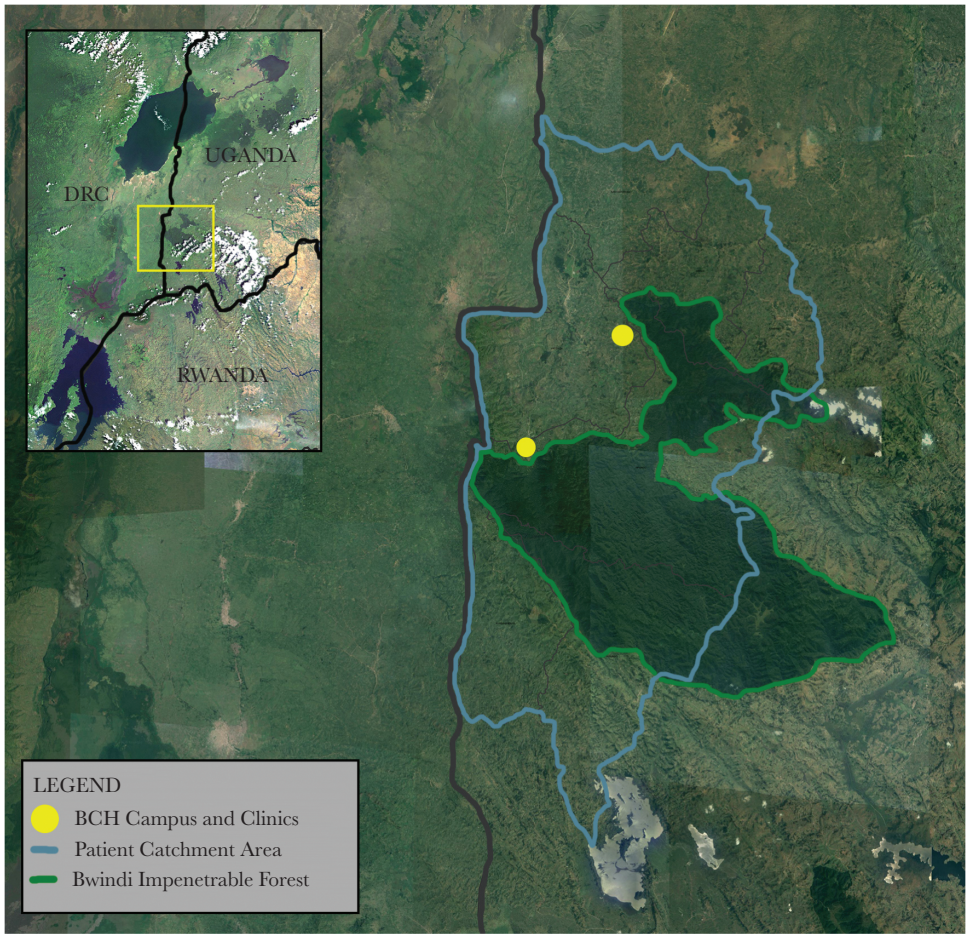

Figure 1. Map of the Bwindi region of southwestern Uganda. Bwindi Community Hospital (BHC) main campus and satellite clinics (yellow) on the edge of the Bwindi Impenetrable Forest. Patients enrolled in the study were residents of Kirundo, Nyabwishenya, Mpungu, Kayonza, Kanungu, Kirima, and Kanyantorogo subcounties in southwestern Uganda.

IgG (KPL, Gaithersburg, MD) at a 1:5000 dilution. The following antigens were used: EBOV, SUDV, and MARV strain Musoke recombinant virus GPs (IBT Bioservices, Gaithersburg, MD) and Bundibugyo virus (BDBV) strain Uganda 2007 recombinant, partial virus envelope GP1 (Ile 33–Gln 304; Acro Biosystems, Newark, DE). Antigens were produced in mammalian cells.

Western blots were validated using convalescent serum from a human who was vaccinated with a recombinant vesicular stomatitis virus (VSV)–based vaccine that expressed an EBOV GP (VSV-EBOV) after suspected EBOV exposure [27], convalescent serum from a MARV-infected nonhuman primate, rabbit anti-SUDV GP polyclonal antibody (IBT Bioservices), and convalescent serum from a rhesus macaque experimentally infected with BDBV [28]. A negative control pool of serum specimens from 20 Ugandan individuals who presented to the Bwindi Community Hospital with respiratory symptoms and showed no filovirus seroreactivity on Western blots, as well as a negative control pool of serum specimens from 5 individuals from other African countries, were used as negative controls. In addition, 15 serum samples collected from volunteer blood donors at the Stanford Blood Center (Palo Alto, CA) were used as negative controls during assay development.

Blots were considered positive for virus if a band of the correct size could be visualized for the GP_1 protein of EBOV, SUDV, and MARV (approximately 150 kDa; Supplementary Figure 1 and 2) and the partial GP_1 protein of BDBV (approximately 60 kDa; Supplementary Figure 3) after visual comparison to positive and negative control samples. A conservative approach to interpretation of Western blot band results was taken in that samples were tested in duplicate and were categorized by 2 independent researchers as positive, negative, or indeterminate, based on visualization of appropriate sized bands.

Statistical Analyses

Individuals with positive and negative samples were used in risk factor analyses and period prevalence calculations; individuals with indeterminate results due to faint bands were excluded (sample sizes used in statistical analyses were as follows: 300 for SUDV analyses, 301 for EBOV analyses, 303 for BDBV analyses, and 331 for MARV analyses). Period prevalence and 95% binomial exact

confidence intervals (CIs) were calculated for demographic groups for each filovirus species. When period prevalence was 0, 1-sided, 97.5% binomial exact CIs were calculated.

All demographic factors, including age, tribe, and livelihood (Table 1), were evaluated for associations with animal contact behaviors (Tables 2 and 3), using bivariate analyses to assess confounding. Associations between filovirus seropositive status and all individual risk factors in Tables 1–3 were initially evaluated by the 2-sided Fisher exact test or the χ^2 test as appropriate, and associations were measured by odds ratios (ORs). Multiple exposures were common, and some behavior categories were associated with each other; therefore, adjustment for various risk factors in multivariable analyses was done sequentially, with priority given to those with a higher OR on crude analysis, those that were highly significant, and those factors with previous evidence based on other investigations.

Multivariable logistic regression was then used to assess the association of risk factors with filovirus exposure for variables that were significant on bivariate analysis ($P < .1$). Separate logistic models were generated for each filovirus species to account for variation in wildlife reservoirs and the risk of transmission to susceptible people. Multivariable logistic regression was also used to assess the association between demographic and livelihood factors and wild animal contact. Variables were included if they significantly improved model fit, based on the likelihood ratio test ($P < .1$), while minimizing the Akaike information criterion. Overall model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test [29]. All statistical analyses were performed using Stata (StataCorp, College Station, TX). Network analyses to generate a wildlife taxa-interface network display (Figure 2) were conducted in the network analysis platform Gephi, using the force-directed algorithm ForceAtlas2 [30].

Ethical Standards

All procedures contributing to this work complied with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Table 1. Sudan Virus (SUDV), Ebola Virus (EBOV), and Bundibugyo virus (BDBV) Prevalence, by Demographic Characteristic, Among Individuals With Wildlife Contact Who Presented to Healthcare Facilities in the Bwindi Region of Uganda During March–June 2013

	SUDV			EBOV			BDBV		
Characteristic	No. Pos	No. Neg	Period Prevalence (95% CI[a])	No. Pos	No. Neg	Period Prevalence (95% CI[a])	No. Pos	No. Neg	Period Prevalence (95% CI[a])
Ethnic group									
Bakiga	11	267	3.96 (1.99–6.97)	14	263	5.05 (2.79–8.33)	23	261	8.10 (5.21–11.90)
Batwa	2	12	14.29 (1.80–42.81)	1	14	6.67 (.17–31.95)	2	11	15.38 (1.92–45.45)
Sex									
Male	7	133	5.00 (2.03–10.03)	9	130	6.67 (3.00–11.94)	12	129	8.51 (4.47–14.39)
Female	7	142	4.70 (1.9–9.43)	7	143	4.67 (1.90–9.38)	13	137	8.67 (4.70–14.39)
Age group									
0–9	1	32	3.03 (.08–15.76)	1	31	3.13 (.07–16.22)	5	25	16.67 (5.64–34.72)
10–19	2	62	3.13 (.38–10.83)	5	58	7.94 (2.63–17.56)	10	55	15.38 (7.63–26.48)
20–29	3	58	4.92 (1.03–13.71)	2	60	3.23 (.39–11.17)	2	62	3.13 (.38–10.48)
30–39	2	41	4.65 (.57–15.81)	2	42	4.55 (.55–15.47)	1	44	2.22 (.06–11.77)
40–49	0	24	0.00[b] (.00–14.25)	3	20	13.04 (2.78–33.59)	1	25	3.85 (.09–19.63)
50–59	2	22	8.33 (1.03–27.00)	3	24	11.11 (2.35–29.16)	0	26	0.00[b] (.00–13.23)
60–69	0	15	0.00[b] (.00–21.80)	0	17	0.00[b] (.00–19.51)	1	16	5.88 (.15–28.69)
≥70	1	14	6.67 (.17–31.95)	0	15	0.00[b] (.00–21.80)	4	10	28.57 (8.39–58.10)
Primary occupation									
Housework	12	224	5.08 (2.65–8.71)	13	224	5.49 (2.95–9.20)	19	227	7.72 (4.71–11.80)
Crop farming	12	228	5.00 (2.61–8.57)	14	228	5.79 (2.89–9.01)	19	230	7.63 (4.66–11.65)
Livestock farming	9	196	4.39 (2.03–8.07)	11	193	5.39 (2.72–9.44)	14	197	6.64 (3.67–10.88)
Hunting	1	0	100.00 (.00–97.50)	0	1	0.00[b] (.00–97.50)	0	1	0.00[b] (.00–97.50)
Working in BINP	0	5	0.00[b] (.00–52.18)	0	5	0.00[b] (.00–52.18)	0	5	0.00[b] (.00–52.18)
Trading	1	4	20.00 (.51–71.64)	0	5	0.00[b] (.00–52.18)	0	5	0.00[b] (.00–52.18)
Business	0	6	0.00[b] (.00–45.93)	0	6	0.00[b] (.00–45.93	0	6	0.00[b] (.00–45.93)
Overall	14	286	4.67 (2.57–7.71)	16	285	5.32 (3.06–8.46)	27	276	8.91 (5.95–12.70)

Indeterminate test results were excluded from analyses.

Abbreviation: BINP, Bwindi Impenetrable National Park; CI, confidence interval; Neg, negative; Pos, positive.

[a]Data are 95% binomial exact CIs, unless otherwise indicated.

[b]One-sided 97.5% binomial exact CIs were calculated.

Table 2. Results of Bivariate Analyses for Risk Factors for Exposure to Ebola Virus (EBOV)

Risk Factor[a]	Seroprevalence in Exposed Group, Proportion (%)	Seroprevalence in Nonexposed Group, Proportion (%)	OR (95% CI)	*P*
Consumed wildlife[b]				
Primate	1/8 (12.5)	15/285 (5.3)	2.57 (.30–22.27)	.365
Bat	…	…	…	>.999
Rodent	2/13 (15.4)	14/282 (5.0)	3.48 (.70–17.23)	.152
Duiker	6/72 (8.3)	10/223 (4.5)	1.94 (.68–5.53)	.217
Porcupine[c]	1/7 (18.7)	15/288 (5.2)	3.03 (.34–26.83)	.326
Wild pig	6/63 (9.5)	10/232 (4.3)	2.34 (.82–6.70)	.114
Hippopotamus	4/57 (7.0)	10/193 (5.2)	1.35 (.30–4.92)	.618
Civet	…	…	…	>.999
Any	11/115 (9.6)	5/180 (2.8)	…	
Contacted wildlife that raided crops				
Primate	3/68 (4.4)	11/183 (6.0)	0.73 (.13–2.89)	.765
Bat	1/12 (8.3)	13/239 (5.4)	1.53 (.03–11.97)	.514
Rodent (all species)	14/223 (6.3)	0/28 (0.0)	…	>.999
Rodent (cane rat)	0/1 (0.0)	14/253 (5.5)	…	>.999
Duiker	1/5 (20.0)	13/246 (5.3)	3.78 (.07–37.33)	.280
Porcupine[c]	1/2 (50.0)	13/249 (5.2)	9.57 (.15–191.09)	.151
Wild pig	1/5 (20.0)	13/246 (5.3)	3.78 (.07–37.33)	.280
Civet	…	…	…	>.999
Any	14/233 (6.0)	0/18 (0.0)	…	
Touched/handled wildlife				
Primate	0/17 (0.0)	16/279 (5.7)	…	>.999
Bat	0/1 (0.0)	16/295 (5.4)	…	>.999
Rodent (all species)	15/210 (7.1)	1/86 (1.2)	6.14 (.91–261.51)	.049
Rodent (cane rat)	1/6 (16.7)	13/245 (5.3)	3.14 (.06–28.90)	.319
Duiker	3/16 (18.8)	13/280 (4.6)	4.03 (.67–16.83)	.065
Porcupine[c]	1/6 (16.7)	15/290 (5.2)	3.22 (.06–29.19)	.311
Wild pig	0/4 (0.0)	16/292 (5.5)	…	>.999
Civet	0/1 (0.0)	16/295 (5.4)	…	>.999
Any	15/212 (7.1)	1/84 (1.2)	…	
Contacted wildlife in forest				
Primate	0/3 (0.0)	16/292 (5.5)	…	>.999
Bat	0/1 (0.0)	16/294 (5.4)	…	>.999
Rodent	1/8 (12.5)	15/287 (5.2)	2.39 (.05–19.88)	.382
Wild birds[d]	2/3 (66.7)	12/248 (4.8)	13.78 (1.03–128.96)	.024
Duiker	0/2 (0.0)	16/293 (5.5)	…	>.999
Porcupine[c]	…	…	…	
Wild pig	0/2 (0.0)	16/293 (5.5)	…	>.999
Civet	0/1 (0.0)	16/294 (5.4)	…	>.999
Any	2/14 (14.3)	12/237 (5.1)	…	

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Having wildlife in homes, having wildlife near homes, having wildlife close to water source, sharing fruits/vegetables with wildlife, being injured by wildlife, finding dead wildlife, hunting wildlife, and consuming wildlife were not significantly associated with EBOV seropositivity for any wildlife species.

[b]Includes both current and past wildlife consumption.

[c]Porcupines are reported separately from rodents because porcupines were not considered to be in the same animal category as smaller rodent species by local study participants.

[d]Contact with wild birds, while significant on bivariate analysis, did not have a large enough sample size to evaluate as a risk factor in a multivariable analysis.

RESULTS

Detection of Previous Exposure to SUDV, EBOV, and BDBV

Febrile patients in this study did not exhibit any signs of hemorrhagic fever and no patients reported having suffered a hemorrhagic illness previously. Diagnoses provided by medical facilities included, malaria, pneumonia, typhoid fever, respiratory infection, urinary tract infection, gastritis, brucellosis, and tuberculosis. All patients had negative results on consensus PCR tests of blood and oral swab specimens for detection of filoviruses. Among study participants, 14 of 300 were seropositive for SUDV, 16 of 301 were seropositive for EBOV, and 27 of 303 were seropositive for BDBV by conventional Western blot (Table 1). Seropositivity to MARV was not detected. One sample was positive for both EBOV and SUDV, and 1 was positive for both EBOV and BDBV. Serologic results for SUDV, EBOV, and BDBV were not significantly correlated with each

Table 3. Results of Bivariate Analyses for Risk Factors for Exposure to Sudan Virus (SUDV)

Risk Factor[a]	Seroprevalence in Exposed Group, Proportion (%)	Seroprevalence in Nonexposed Group, Proportion (%)	OR (95% CI)	*P*
Hunted wildlife				
Primate	4/7 (57.1)	8/242 (3.3)	39.0 (7.46–204.00)	.001
Bat	0/1 (0.0)	12/248 (4.8)	...	>.999
Rodent	1/3 (33.3)	11/246 (4.5)	7.46 (.13–100.13)	.173
Duiker	1/3 (33.3)	11/246 (4.5)	7.46 (.13–100.13)	.173
Porcupine[b]	0/1 (0.0)	12/248 (4.8)	...	>.999
Wild pig	1/3 (33.3)	11/246 (4.5)	7.46 (.13–100.13)	.173
Civet	...	...	...	
Any	4/9 (44.4)	8/240 (3.3)	...	
Consumed wildlife[c]				
Primate	0/6 (0.0)	13/288 (4.5)	...	>.999
Bat	...	...	...	
Rodent (all species)	3/14 (21.4)	10/280 (3.6)	6.00 (.95–26.81)	.029
Rodent (cane rat)	3/11(27.3)	9/237 (4.0)	7.18 (1.08–34.20)	.021
Duiker	5/70 (7.1)	8/224 (3.6)	2.00 (.50–7.18)	.229
Porcupine[b]	1/7 (14.3)	12/287 (4.2)	3.42 (.07–30.21)	.296
Wild pig	4/64 (6.25)	9/230 (3.9)	1.60 (.35–5.93)	.494
Hippopotamus	4/60 (6.67)	8/188 (4.3)	1.57 (.33–6.09)	.491
Civet	0/1 (0.0)	13/293 (4.4)	...	>.999
Any	5/115 (4.3)	8/179 (4.5)	...	
Contacted wildlife that raided crops				
Primate	6/71 (8.5)	6/178 (3.4)	2.51 (.64–9.68)	.111
Bat	0/12 (0.0)	12/237 (5.1)	...	>.999
Rodent (all species)	11/222 (5.0)	1/27 (3.7)	1.34 (.18–59.68)	>.999
Rodent (cane rat)	1/2 (50.0)	11/249 (4.4)	11.31 (.17–227.25)	.131
Duiker	0/5 (0.0)	12/244 (4.9)	...	>.999
Porcupine[b]	0/3 (0.0)	12/246 (4.9)	...	>.999
Wild pig	1/5 (20.0)	11/244 (4.4)	4.43 (.09–44.54)	.248
Civet	...	...	...	
Any	11/232 (4.7)	1/17 (5.9)	...	
Touched/handled wildlife				
Primate	3/16 (18.8)	10/278 (3.6)	5.21 (.83–22.89)	.039
Bat	...	...	...	
Rodent (all species)	11/212 (5.2)	2/82 (2.4)	2.13 (.48–20.12)	.526
Rodent (cane rat)	2/7 (28.6)	10/241 (4.1)	6.89 (.61–42.55)	.056
Duiker	1/14 (7.1)	12/280 (4.3)	1.67 (.04–12.87)	.486
Porcupine[b]	1/6 (16.7)	12/288 (4.2)	4.0 (.08–37.09)	.264
Wild pig	0/4 (0.0)	13/290 (4.5)	...	>.999
Civet	0/1 (0.0)	13/293 (4.4)	...	>.999
Any	11/213 (5.2)	2/81 (2.5)	...	

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Having wildlife in homes, having wildlife near homes, having wildlife close to water source, contacting wildlife in the forest, sharing fruits/vegetables with wildlife, being injured by wildlife, and finding wildlife dead were not significantly associated with SUDV seropositivity for any wildlife species.

[b]Porcupines are reported separately from rodents because porcupines were not considered to be in the same animal category as smaller rodent species by local study participants.

[c]Includes both current and past wildlife consumption.

other ($R^2 = 0.03$ for the correlation between SUDV and EBOV, $R^2 = -0.002$ for the correlation between EBOV and BDBV, and $R^2 = 0.002$ for the correlation between SUDV and BDBV), indicating that there was limited cross-reactivity among known filovirus species on these serological assays.

Wild Animal Contact in Study Community

Over ninety-nine percent of patients reported some form of contact with wildlife. The wildlife species most commonly contacted were rodents (Rodentia species; 99.6% had indirect contact, and 86.4% had direct contact), followed by primates (Primate species; 62.3% and 15.5%, respectively), bats (Chiroptera species; 67.0% and 5.5%, respectively), duiker (*Cephalophus* species, 5.5% and 29.0%, respectively), wild pig (*Potamochoerus larvatus*; 8.1% and 26.6%, respectively), porcupine (*Atherurus africanus*; 3.3% and 4.1%, respectively), and civet (*Civettictis civetta*; 0.7% and 0.7%, respectively). Contact due to the presence of wild animals in and around peoples'

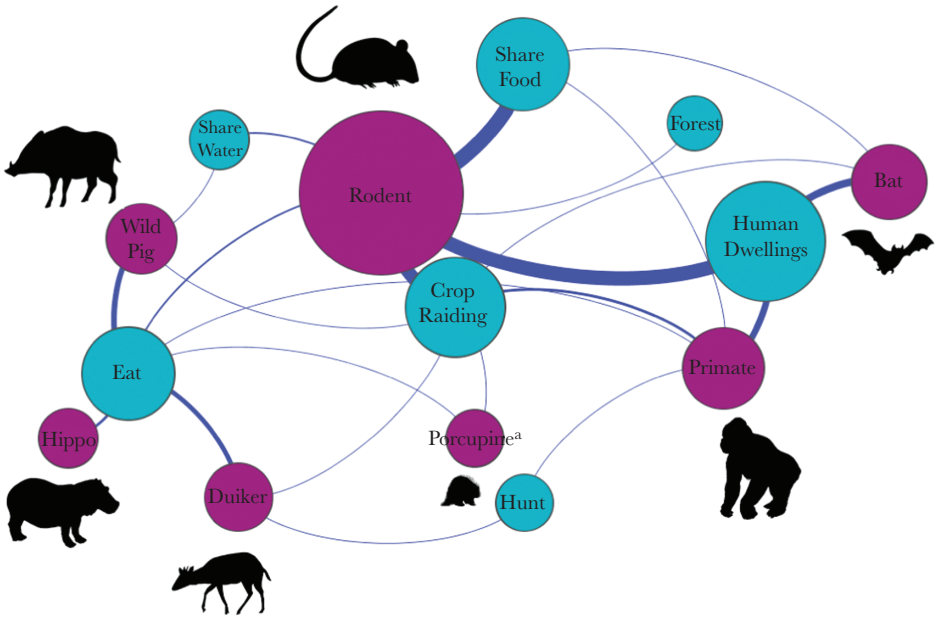

Figure. 2. Epidemiologic bipartite network map showing disease transmission interfaces shared by humans and wildlife in the Bwindi region of southwestern Uganda. For transmission interfaces (blue), the node size is proportionate to the number of people reporting direct or indirect contact with wildlife at each interface. For wildlife taxa (purple), the node size is proportionate to the number of people reporting contact with each respective wildlife taxa. Highly connected and more-central interfaces represent greater opportunities for contact with multiple wildlife host taxa and therefore present greater chances for humans to encounter zoonotic pathogens. This network was created using data on contact between humans and animals that were collected from 331 febrile individuals presenting to medical facilities in the Bwindi region. [a]Porcupines are reported separately from rodents because porcupines were not considered to be in the same animal category as smaller rodent species by local study participants.

homes was widespread (99% [270 of 274 patients]), and contact with animals that were raiding crops (93% [255 of 274]) and sharing food items (91% [296 of 325]) was also common. Contact with animals through bushmeat hunting was less commonly reported (5% [13 of 274 patients]; Figure 2).

On multivariable analysis, men were more likely than women to consume wildlife (OR, 3.18; 95% CI, 1.09–9.32; $P = .035$) and to touch duikers (OR, 6.62; 95% CI, 1.55–28.21; $P = .011$). People working as park personnel were 9.5 times as likely to consume wildlife than people with other occupations (95% CI, 1.43–63.25; $P = .020$). People of the Batwa tribe were also 9.7 times as likely to consume wildlife (95% CI, 2.08–44.84; $P = .004$), 7.1 times as likely to hunt wild animals (95% CI, 1.20–41.55; $P = .031$), and 9.5 times as likely to touch a duiker (95% CI, 1.71–52.76; $P = .010$) than people of the Bakiga tribe.

Wildlife Contact Associated With EBOV Seropositivity

Results of our multivariable model showed that people with a history of touching duikers were 5.6 times as likely to be seropositive for EBOV than people who reported no contact with duikers (95% CI, 1.23–25.17; $P = .026$; Table 2 and Table 4). Age group was included in the model because a history of touching duikers was found to increase with age. The log odds of EBOV seropositivity was predicted by use of the following logistic model: $\ln[P(x)/1 - P(x)] = -2.64 - 0.11(A) + 1.72(T_d)$, where $P(x)$ denotes the probability of a person being seropositive for EBOV (Hosmer-Lemeshow model goodness-of-fit $\chi^2 = 76.38$; $P = .785$), A denotes age in years, and T_d denotes a history of touching a duiker.

Wildlife Contact Associated With SUDV Seropositivity

Results of our multivariable model showed that people reporting a history of hunting primates were 37.5 times as likely to have evidence of past exposure to SUDV than people who did not report hunting primates (95% CI, 6.20–226.41; $P < .001$; Table 4). Also, people with a past history of touching or eating cane rats (*Thryonomys swinderianus*) were 10.7 times as likely to show serologic evidence for past exposure to SUDV than people who did not report touching or eating cane rats (95% CI, 1.95–58.15; $P = .006$; Table 4). Tribal affiliation was included in the model because being of Batwa ethnicity was significantly associated with hunting wild animals. On bivariate analyses, both touching and eating cane rats were independently associated with SUDV seropositivity (Table 3) but, when combined, were a better predictor of risk of exposure, and they were therefore incorporated as a combined variable into the multivariable model. The log odds of SUDV seropositivity was predicted by use of the following logistic model: $\ln[P(x)/1 - P(x)] = -3.81 + 1.79(T) + 2.37(C_{te}) + 3.62(P_h)$, where $P(x)$ denotes the probability of a person being seropositive for SUDV (Hosmer-Lemeshow model goodness of fit $\chi^2 = 0.42$; $P = .810$), T denotes tribe, C_{te} denotes a history of touching or eating a cane rat, and P_h denotes a history of hunting primates.

Table 4. Odds Ratios and 95% Confidence Intervals (CIs) for Risk Factors Associated With Filovirus Seropositivity Based on Final Multivariable Analyses

Filovirus, Risk Factor	Seroprevalence of Risk Factor, Exposed Group, Proportion (%)	Seroprevalence of Risk Factor, Nonexposed Group, Proportion (%)	OR (95% CI)	*P*
Ebola virus[a]				
Touched duiker	3/16 (18.8)	13/280 (4.6)	5.57 (1.23–25.17)	.026
Sudan virus[b]				
Hunted primate	4/7 (57.1)	8/242 (3.3)	37.47 (6.20–226.41)	<.001
Touched/consumed cane rat	3/12 (25.0)	9/235 (3.8)	10.65 (1.95–58.15)	.006

[a]Age was associated with touching a duiker. Therefore, age was included in the final multivariable model.

[b]Being of the Batwa tribe was associated with hunting primates. Therefore, tribe was included in the final multivariable model.

No variables describing contact between humans and wildlife were significant on bivariate analysis for BDBV.

DISCUSSION

We provide evidence for previous exposure to filoviruses among people in the Bwindi region of southwestern Uganda. Our serologic findings fill a gap in geographic exposure data for the region, as outbreaks of EHF have occurred in neighboring districts of Bundibugyo and Kibaale and across the border in the DRC [9, 31–33], and MHF has occurred in Kabale and Maramagambo forests [9, 34, 35].

It is unlikely that clinically significant EHF cases have occurred and gone unreported in the Bwindi region, given the accessibility of Bwindi Community Hospital and its associated satellite clinics. Additionally, cases of filovirus-associated hemorrhagic fever in mountain gorillas, a species heavily monitored, highly contacted by people, and susceptible to filovirus infection, have not been reported. Identification of IgG antibodies to filovirus GPs in humans presenting to clinics in Bwindi therefore could be due to asymptomatic infection with SUDV, EBOV, or BDBV and/or infection with serologically cross-reactive low-pathogenicity or nonpathogenic undiscovered filoviruses that also have wildlife hosts.

Previous studies suggest that EBOV infections can by asymptomatic or minimally symptomatic [36–38]. Seropositivity in people who did not report a history of hemorrhagic fever has been demonstrated in EHF-endemic regions [23, 39], and asymptomatic family members of patients infected with known filovirus species have shown seropositivity [40]. We also cannot rule out the possibility that we detected antigenically similar but as-yet unknown filoviruses. Additional filoviruses are expected to be discovered as diagnostic assays and surveillance activities improve. Past serosurveys using antigens that are more broadly reactive among filoviruses have detected filovirus exposure among humans and wildlife living in areas where no cases of EHF have ever been reported, such as the Aka population of the Central African Republic [22] and fruit bat populations in Bangladesh [41]. We used GP antigens in this study because it is predicted that the antibody response to GP is more virus specific, owing to the larger genetic variability with this protein [42]. Serologic cross-reactivity between filovirus species has however been noted in enzyme-linked immunosorbent assays using recombinant GP antigens, although the highest reactions were uniformly detected against the GP antigen homologous to the correct virus species [43].

Interestingly, no one tested positive for a previous exposure to MARV, even though outbreaks of MARV infection have occurred in close proximity to the Bwindi region. An outbreak of MARV infection occurred in 2012 in Kabale District [44], approximately 26 km from the southern corner of Bwindi Impenetrable Forest. In addition, 2 foreigners who visited caves in Maramagambo Forest in the southern corner of Queen Elizabeth National Park, approximately 80 km from the northern-most corner of Bwindi Impenetrable Forest, exported MARV in 2008 to the United States and the Netherlands [45, 46]. MARV has a documented case-fatality rate between 24%–88%, indicating that there is potential for detection of seropositive survivors [47, 48]. Our lack of detection of MARV-seropositive people in the Bwindi region suggests that exposure to MARV could be rarer than for other filoviruses and that wildlife reservoirs are more restricted in range or habitat.

We found variations in seroprevalence among people engaged in different activities involving wildlife, providing epidemiologic evidence that filoviruses circulating in Bwindi are of wildlife origin. In contrast to similar studies in West and Central Africa, where bushmeat hunting is the major activity leading to human and wild animal contact, we found a broader scope of activities that leads to interaction with wildlife.

Contact with duiker was identified as a risk factor for EBOV exposure. Duikers are frequently eaten by humans in many parts of equatorial Africa and have been previously implicated as incidental hosts for EBOV. Duikers are believed to become infected with EBOV through scavenging nonhuman primate carcasses [13], an activity that is plausible in Bwindi given the prevalence and diversity of nonhuman primate species. Duikers are the most common animal caught in snares in Bwindi Impenetrable National Park, and people directly contact this species through hunting, food preparation, and consumption. Duikers also frequently leave the forest and can be found crop raiding among private gardens.

Hunting primates was identified as a significant risk factor for SUDV seropositivity, consistent with previous findings, which have linked outbreaks of EHF, caused by EBOV, to contact with dead common chimpanzees and/or western lowland gorillas [12, 13]. Interestingly, contrary to previous beliefs that hunting and consumption of primates was almost nonexistent in Uganda [24], we identified several people presenting to Bwindi Community Hospital who reported hunting primates or knowing others in their close community who hunted primates. All hunting of vertebrate species living within Uganda's forest reserves is prohibited by law, and Ugandans generally consider primate meat consumption culturally unacceptable. Hunting of primates, however, may be on the increase in this region because of population growth both within Uganda and through influx of refugees from the DRC, where primate bushmeat consumption is more common.

Contact with cane rats was also identified as a risk factor for SUDV exposure. Rodents have been identified as potential filovirus hosts in previous investigations, but evidence has been weak and they have received less attention as more evidence has pointed toward bats and primates as reservoirs and incidental hosts, respectively. Cane rats are of particular concern as a potential host for infectious diseases because of their proximity to people. They commonly range freely around human dwellings and are also raised as a source of meat in West and Central Africa. In the Bwindi region, cane rats are one of the most commonly eaten wildlife species and are frequently found raiding crops and food storage areas, where people indirectly contact their feces and/or urine.

Finding evidence for filoviruses in Bwindi could have implications not only for the human communities surrounding and using the park but also for the critically endangered mountain gorilla. Human and mountain gorilla health and livelihoods in this region are intimately connected; they are susceptible to the same pathogens, and gorilla tourism supports the local economy, which in turn preserves the forest. A filovirus hemorrhagic fever outbreak in Bwindi could have devastating implications for humans and gorillas alike. Additionally, if unknown filoviruses are circulating in Bwindi, which theoretically could confer some degree of cross-protective immunity, this might help explain why filovirus-associated hemorrhagic fever outbreaks have not been reported to date in this region. A better understanding of the filoviruses circulating in Bwindi, as well as their hosts and/or likely reservoir species, is needed to recognize and better understand any potential threat. We conclude that broader surveillance strategies are needed, moving beyond dead nonhuman primates and the bat species tested to date, to characterize filovirus transmission cycles in Africa.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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