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BRIEF REPORT

Serology and Behavioral Perspectives on Ebola Virus Disease Among Bushmeat Vendors in Equateur, Democratic Republic of the Congo, After the 2018 Outbreak

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After the 2018 Ebola outbreak in Equateur Province, Democratic Republic of the Congo, we conducted behavioral interviews and collected samples from bushmeat vendors and primates in Mbandaka to test for evidence of Ebola virus exposure. Although participants indicated being aware of Ebola, they did not consider themselves at occupational risk for infection. We found antibodies against Zaire ebolavirus in one participant despite no reported history of disease or contact with infected individuals. Our data underline concerns of possible subclinical or undiagnosed Ebola virus infections and the importance and challenges of risk communication to populations who are occupationally exposed to bushmeat.

Keywords. behavior; bushmeat; Ebola virus disease; risk perception; serology.

Since Ebola virus disease (EVD) was first reported in 1976, 6 different species in the Ebolavirus genus have been discovered [1, 2]. Outbreaks were historically small and locally contained until the 2012–2014 West Africa epidemic, which caused over 11 000 deaths. This outbreak spurred an international response, with a significant focus on both emergency health services and scientific research on understanding Ebolavirus transmission, spread, prevention, and the health system failures that lead to EVD propagation [2]. Despite all efforts and lessons learned, EVD continues to be a problem, particularly in the Democratic Republic of the Congo (DRC), which is experiencing its 10th EVD outbreak, 9 of which have been caused by Zaire ebolavirus (EBOV). The North Kivu outbreak has endured despite efforts to vaccinate and treat the populations at risk [2]. Difficulty in containing outbreaks is in part due to armed regional conflict and community resistance and skepticism of both medical personnel and Ebola treatment centers, which has contributed to continued transmission and cases in Eastern DRC.

Although the origin of EBOV remains unclear, mounting evidence indicates that certain bat species may act as reservoirs [1–4]. In some instances, previous outbreaks of EVD have been traced back to confirmed or suspected contact with wild animals, specifically bats and nonhuman primates (NHPs), with the latter potentially serving as an intermediate host [5–7]. Practices such as hunting and butchering of wildlife increase the risk of transmission of zoonotic pathogens, including EBOV. The behavior and exposure of people engaged in the wildlife trade, and their perception of EVD risk, may be key factors influencing spillover events and merits further exploration. Population-wide surveys of EBOV exposure are limited, and therefore increased effort is needed to understand disease dynamics such as asymptomatic and subclinical infections in both non-outbreak and post-outbreak locations [8–10].

To better understand the risk perception, behavior, and EBOV exposure of bushmeat market vendors during an EVD outbreak, we conducted a study in Mbandaka, Equateur Province, DRC after the 2018 EVD outbreak. Data and sample collection occurred 4 months after the official end of the epidemic and included interviews, questionnaires, and biological sample collection from bushmeat vendors and NHPs for EBOV serology and polymerase chain reaction (PCR) testing.

METHODS

Interviews and sampling were conducted in Mbandaka between November 12 and 16, 2018 (Figure 1). Study protocols and materials were reviewed and approved by the Institutional Review Board (IRB) committee of the École de Santé Publique in Kinshasa, DRC, and the IRB and Institutional Animal Care and Use Committee of the University of California, Davis, California.

Bushmeat vendors were enrolled in this study from 2 different markets in Mbandaka. Informed and written consent was obtained from all participants before blood collection and administration of a short questionnaire and a semistructured interview. Questionnaires and interviews aimed to collect

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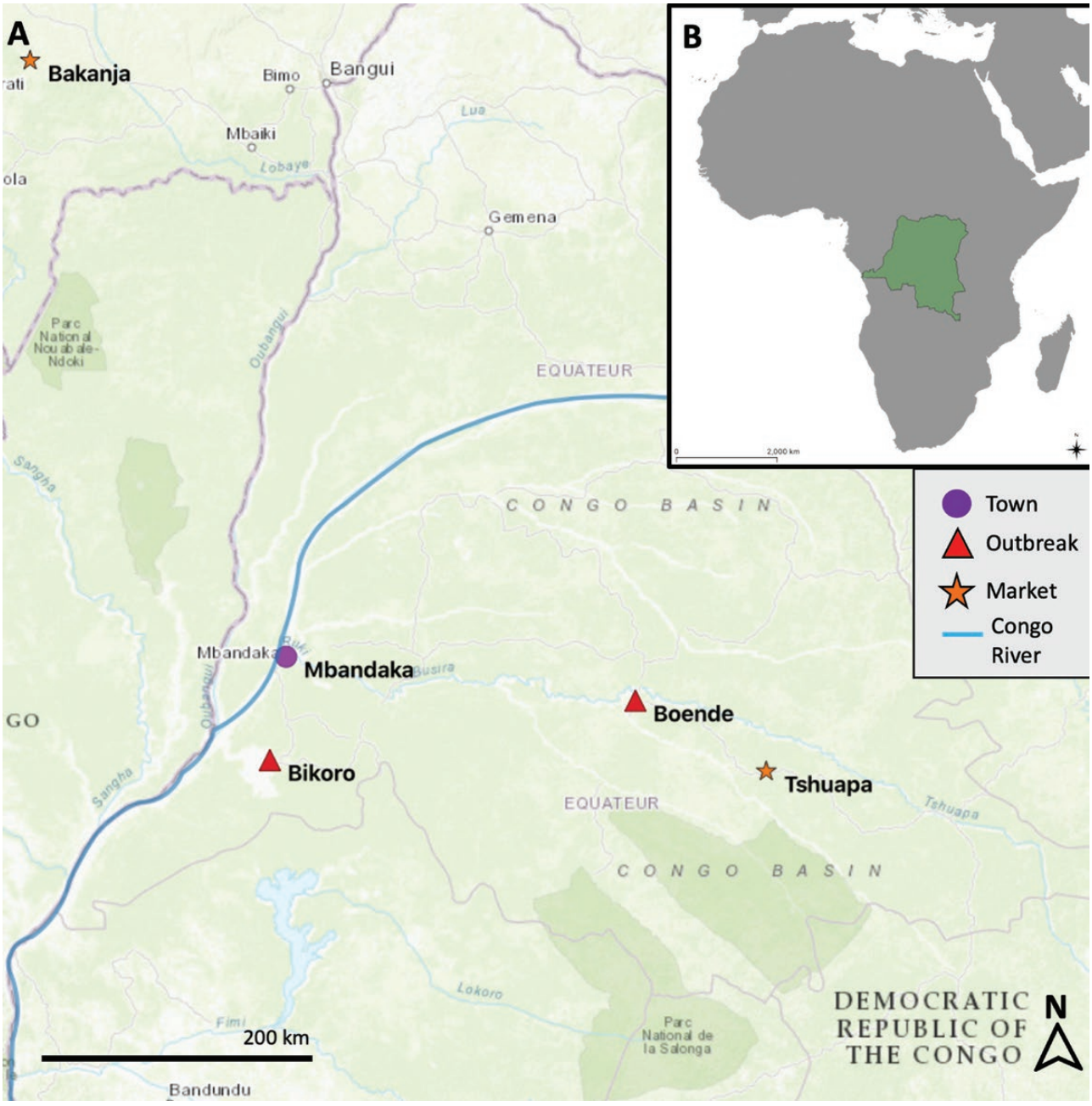

Figure 1. Map showing the study location (purple dot) in relation to previous outbreak sites (red triangles) and sources of bushmeat (yellow stars).

information about participant occupation, and their understanding of, beliefs toward, and previous exposure to EVD, as well as other relevant medical history.

Nonhuman primates were sampled from the same markets as participants, and also from surrounding villages where hunters were selling animals as bushmeat or pets. Samplers wore dedicated but inconspicuous clothing and protected themselves by wearing glasses, a mask and gloves during visits. For the actual sampling disposable personal protective equipment such as a protective gown, mask and gloves were worn. Blood samples were collected from live animals, while samples of liver, lung, spleen, intestine, blood, and oral and rectal swabs were collected from dead animals, as available. All swab and tissue samples, were collected into 2 tubes containing either 500 µL TRIzol or 500 µL VTM. Whole blood samples were taken without transport medium or anticoagulant. These whole blood samples were centrifuged in the field, and separated serum was collected and placed in cryotubes. Serum samples were only available for humans, due to the small volume of whole blood obtained from NHPs. All samples were initially stored in liquid nitrogen and later transferred to a −80°C freezer.

Serum samples were heat inactivated and screened in duplicate by enzyme-linked immunosorbent assay for reactivity to EBOV using the recombinant full-length EBOV GP protein (rGP, 0.25 µg/mL; R&D Systems, Minneapolis, MN). Because of the potential for cross reactivity, reactive samples on the EBOV assay were then differentially screened in duplicate against the other filoviruses using recombinant full-length GP protein for Sudan (SUDV, 0.25 µg/mL; IBT Bioservices, Rockville, MD), Bundibugyo (BDBV, 0.25 µg/mL; IBT Bioservices), Taï Forest (TAFV, synthesized), Bombali (BOMV, synthesized), and Marburg virus (MARV, 0.25ug/mL; IBT Bioservices). Positive controls included polyclonal antibodies raised in rabbits against the EBOV (1:2000, eEnzyme LLC, Gaithersburg, MD), SUDV (1:1000; IBT Bioservices), BDBV (1:2000; Sino Biological, Wayne, PA), RESTV (1:2000; Abcam, Cambridge, MA), TAFV (1:1000; Alpha Diagnostics, San Antonio, TX), BOMV (1:2000) and MARV (1:2000; IBT Bioservices) rGPs. Commercially available negative human serum (1:200; Millipore Sigma, Burlington, MA) was used as a negative control. The endpoint titer of positive samples was determined by 2-fold dilutions using the recombinant full-length GP protein for the virus that had the highest reactivity in the differential screen [11]. A sample was considered reactive when the absorption was higher than 3 times the background (no antigen) or the negative wells (whichever was higher).

Ribonucleic acid was extracted from TRIzol inactivated swabs, whole blood, serum, and tissue samples from humans and NHPs in a BSL2 laboratory using a Zymo Direct-zol RNA kit (Zymo Research Corp, Irvine, CA), followed by reverse transcription using Superscript III (Invitrogen, Carlsbad, CA). Amplifiable RNA was confirmed in all samples by conventional PCR for host Cytochrome B using, as well as to confirm host species identification in nonhuman primates [12]. Samples were screened using 3 assays: (1) a nested filovirus "family level" consensus PCR (cPCR) targeting a 680-bp fragment of the filovirus L gene [1], (2) an Ebolavirus "genus level" cPCR targeting a 187-bp fragment of the NP gene [13]; and (3) a real-time PCR specific for the EBOV virus, targeting the L-gene (Supplement 1) [14].

RESULTS

Nineteen female bushmeat vendors enrolled in the study (9 refusals), provided biological samples and completed a questionnaire, with 13 of them also participating in a more thorough semistructured interview (Table 1, Supplement 2).

Swab and tissue samples were collected from 22 NHPs (13 live, 9 dead), of which 7 were identified as *Cercopithecus ascanius*, 2 as *Cercopithecus nictitans*, 2 as *Allenopithecus nigroviridis*, and one each as *Cercocebus agilis*, *Cercopithecus wolfi*, and *Lophocebus aterrimus*; 8 were not identified beyond the Cercopithecidae family. Blood samples were collected from 4 of these NHPs (Supplement 3).

Questionnaires and Interviews

In the questionnaires, none of the vendors reported being knowingly exposed to or diagnosed with EVD, being closely associated with a person who was diagnosed with EVD, or being vaccinated against EBOV. No participant believed that their occupation in the bushmeat trade put them at risk of contracting the disease, and all reported regular daily contact with wild animal meat.

In interviews, all 13 participants reported hearing that EBOV originated from wild animals or bushmeat and most (85%) had heard that the animal implicated in the 2018 Mbandaka outbreak was a monkey. All reported hearing some kind of educational message about EVD, but many participants harbored skepticism about the existence of EBOV, because they either didn't "*see it with their own eyes*," had continued eating bushmeat and did not get sick, considered it an illness of "*elsewhere*" or of the "*interior*" (ie, other provinces where there have been multiple EVD outbreaks), or claimed that EBOV did not affect people in Mbandaka.

Regardless of some initially expressed skepticism, most participants (62%) did admit to believing EVD was a real illness when asked directly. Twelve vendors continued to sell bushmeat during the 2018 outbreak. Nine of the 12 reported that their sales were negatively affected in some way, such as lower prices, some species being more difficult to sell than others, or generally decreased demand (23% each). In particular, 3 of them reported an inability to sell monkey and 3 reported a general decrease in bushmeat sales. Although all participants heard that bushmeat was the source of the EVD outbreak, all but one vendor continued eating bushmeat during the outbreak.

All participants reported receiving EVD prevention and education messages, with hand washing being the recommendation most frequently recalled (85%). Participants reported more frequent hand washing before and after both eating and using the toilet as the primary prevention effort they took against exposure to EBOV. Some vendors (38%) reported that a water receptacle for the purpose of hand washing was placed outside the market during this time and stressed the importance of using soap.

Based on semi-structured interviews, vendors reported that they thought the 2018 epidemic was less severe than the one in 2014. Common things vendors said about the first outbreak was that it was "*strong*," many people died, and the community was very scared. They indicated that the 2018 outbreak was largely ignored by most people in Mbandaka. Although some participants reported having to sell bushmeat in secret, others continued to sell in the market and reported no regulatory controls were enforced by authorities. Other participant behaviors and beliefs related to EVD are summarized in Table 1.

Table 1. Summary of Beliefs and Behaviors Surrounding Ebola Virus Disease Outbreaks

Participant No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Believes EVD is a real illness[a]	X	✓	✗	✗	✓	✓	✓	N/A	✓	✓	✓	✗	✓
Information Source About the outbreak													
Radio	N/A	N/A	✓	✓	✓	✓	✓	N/A	N/A	✗	✓	✓	N/A
Doctors	✓	N/A	✓	✓	✓	✓	N/A	N/A	✓	✓	N/A	N/A	✗
Word of mouth	N/A	N/A	✓	N/A	✓	N/A	✓	✓	✓	N/A	✓	✓	✓
Church	✓	✓	✓	N/A	N/A	✓	✗	N/A	N/A	N/A	N/A	N/A	
Knew/heard of someone with or cases of EVD	N/A	✓	✓	✗	✓	✓	✗	✓	✓	✓	N/A	✗	✓
Knowledge About Source of EVD													
Wild animals	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Monkey	N/A	✓	✓	✓	✓	✓	✓	✓	✓	✓	N/A	✓	✓
Reasons for Skepticism													
Not seen with own eyes	✓	✗	✓	✓	✗	N/A	✓	✓	N/A	✓	✓	✓	✗
Ate bushmeat, did not get sick	N/A	✗	N/A	✓	✗	✓	✓	N/A	✓	N/A	✓	✓	✓
EVD is illness from elsewhere	N/A	✗	N/A	N/A	✗	N/A	N/A	✓	N/A	N/A	N/A	✓	N/A
Experiences During Outbreaks													
Continued to sell bushmeat	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sold for lower price	N/A	N/A	N/A	✓	N/A	✓	N/A	N/A	✓	✓	✓	N/A	N/A
Inability to sell certain species	N/A	✓	N/A	N/A	N/A	✓	N/A	N/A	N/A	N/A	✓	✓	N/A
Lower demand for bushmeat	N/A	✓	✓	✓	✓	✓	✓	✓	✓	✓	N/A	✓	✓
Continued eating bushmeat during outbreak	✓	N/A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Epidemic had an effect bushmeat sales	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Preventative Measures During Outbreaks													
Handwashing to prevent EVD	✓	✓	✓	✓	✓	✓	N/A	✓	✓	✓	N/A	✓	✓
Handwashing before/after toilet	✓	N/A	✓	✓	✓	✓	N/A	✓	✓	✓	N/A	N/A	✓
Handwashing before/after eating	N/A	✓	✓	✓	✓	✓	N/A	✓	✓	✓	N/A	N/A	N/A
Handwashing before/after leaving market or after selling/handling meat	✓	N/A	N/A	N/A	✓	✓	N/A	N/A	N/A	N/A	N/A	✓	✓
Teaching children to wash their hands	N/A	✓	N/A	N/A	✓	✓	N/A	✓	N/A	✓	N/A	N/A	N/A
Water basin placed at/outside market	✓	N/A	N/A	N/A	✓	✓	N/A	✓	✓	✓	N/A	✓	✓
Soap important	N/A	✓	✓	N/A	✓	✓	N/A	N/A	✓	N/A	N/A	N/A	N/A
Comparison of 2014 and 2018 Outbreaks													
Heard 1st (2014) outbreak originated in Boende	✗	N/A	N/A	N/A	N/A	N/A	✓	✗	✓	✓	✓[b]	✗	✓[b]
Heard 2nd (2018) outbreak originated in Bikoro	N/A	✓	✓	✓	✓	N/A	✓	✓	✓	✓	✓	✓	✓
2014 outbreak was strong	N/A	N/A	N/A	N/A	✓	✓	N/A	N/A	✓	✓	✓	N/A	N/A
2018 outbreak was weaker than 2014	N/A	N/A	N/A	N/A	✓	✓	✗	N/A	✓	✓	✓	N/A	N/A
During the 2014 people were scared	N/A	N/A	N/A	✓	✓	✓	N/A	N/A	✓	N/A	N/A	N/A	N/A
2018 outbreak was largely ignored	N/A	N/A	N/A	✓	N/A	✓	✗	N/A	✓	N/A	N/A	N/A	N/A
Only heard/mentioned one outbreak (the 2nd)	✓	✓	✓	✗	✗	✗	✗	✓	✗	✗	✓[b]	✓	✓[b]
Regulations													
Said there were bans on bushmeat during (at least) one of the Ebola outbreaks	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗

Abbreviations: EVD, Ebola virus disease; N/A, no answer was given or question not asked

✓ Indicates a Yes answer.

✗ Indicates a No answer.

[a]Direct question regardless of any previously expressed skepticism.

[b]Heard of an outbreak in Boende but associated it with Cholera.

Polymerase Chain Reaction and Serology

Filovirus RNA was not detected in any of the samples from either humans or NHPs via either consensus or real-time PCR (Supplements 2 and 3).

Antibodies (IgG) against EBOV were found in the serum of one of the 19 bushmeat vendors (5%), with a titer of 1:800; none were detected in the NHP serum samples. The antibody-positive individual reported during the interview that she had heard of EVD but did not pay much attention to the 2018 outbreak, as she considered it not very serious. She was not aware of anyone in her family having contracted EVD and did not believe herself to have been exposed. She continued butchering and selling various types of bushmeat throughout the outbreak, and also prepared and consumed bushmeat herself. She reported that the meat she sells comes from local forests, in areas such as Tshuapa, Mayi Pembe, and Bakandja, but not from Bikoro, which was the epicenter of the 2018 EVD outbreak in Equateur province (Figure 1).

When questioned about prior medical history, she reported having received a cholera diagnosis in 2015, although

it is unclear from the interview whether this was a clinical or laboratory diagnosis. During her bout of cholera, she had returned home from the market and woke up from sleep later that night vomiting. After vomiting again and feeling dehydrated, she went to the hospital where she was diagnosed with cholera, was given intravenous fluids, and then discharged. She noted that other people she was hospitalized with at the time died, although did not provide specifics about which illnesses they had.

DISCUSSION

Although many of the vendors present at the markets had reservations about participating in the study, the responses obtained from subjects highlight some important points and confirm observations from West Africa that attempts to control bushmeat sales and implementing regulations on eating and selling bushmeat have eroded public confidence, increased skepticism, and have unintended consequences for communities [15]. Although all subjects reported having heard about EBOV, and that the virus originates in wild animals, none of them considered themselves as being at any occupational risk of infection. This perception of low risk in their occupation could be a reflection of occupational bias, because members of the bushmeat trade might want to protect their business, as opposed to true disavowal of risk, because the majority of the vendors interviewed did believe in the existence of EVD.

Misinformation and the unintended consequences of bushmeat regulations and public health messaging is a difficult issue to address. Educational campaigns and warnings with respect to the risks of bushmeat consumption would be most essential in-between outbreaks to prevent the initial spillover events that could transcend into large-scale human outbreaks, especially given that once an outbreak has begun, human to human transmission is the primary means of propagation. Once an outbreak has begun, it is worth re-evaluating strict top-down regulations and bans on bushmeat given the underground networks that develop. It is also worth considering that the majority of participants heard messages about hand washing and followed these precautions during the active outbreak phase in 2018, even if they did not consider this epidemic serious. It is also important to recognize that people in an area such as Mbandaka, who have experienced more than one EVD outbreak, may become less susceptible to the same public health messaging used in the first outbreak event.

We detected one individual with seroreactivity to EBOV in a group of 19 bushmeat vendors (5%). Despite 2 previous EVD outbreaks in the area and the individuals being at an elevated occupational risk of contracting zoonotic diseases, none of them had reported any history of or contact with EVD. The serum of the EBOV antibody positive individual did not show any cross-reactivity with other known filoviruses, so we assume an immune response against pathogenic EBOV. Consequently the individual lived through either a subclinical or an atypical course of infection, that was potentially misdiagnosed. Considering the frequent occurrence of other diseases such as malaria, shigellosis, typhoid fever, and cholera (outbreak 2016/2017 in Equateur) in DRC, an atypical EBOV infection may go undetected, and previous reports of EBOV antibodies in individuals with no known EVD history or contact support that [9, 10]. A number of factors may explain the presence of atypical or subclinical infections, including route and dose of exposure, competency of the host's immune system, and previous exposure to a related virus providing some cross-protection [8, 9, 14].

CONCLUSIONS

Further research on a larger scale encompassing both serological and behavioral components of EBOV and EVD, especially among at-risk populations in DRC would help elucidate the issues highlighted here.

Supplementary Data

Supplementary materials are available at Open Forum 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.

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