Bombali Virus in *Mops condylurus* Bats, Guinea

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In 2018, a previously unknown Ebola virus, Bombali virus, was discovered in Sierra Leone. We describe detection of Bombali virus in Guinea. We found viral RNA in internal organs of 3 Angolan free-tailed bats (*Mops condylurus*) trapped in the city of N'Zerekore and in a nearby village.

In 2018, a new species of the genus *Ebolavirus* (family *Filoviridae*), Bombali virus (BOMV), was discovered in Sierra Leone (*1*). The virus was detected in oral and rectal swab specimens from 2 free-tailed bat species, *Chaerephon pumilus* (little free-tailed bat) and *Mops condylurus* (Angolan free-tailed bat). Both bat species are widespread in Africa, and their ranges include countries where human Ebola virus disease (EVD) outbreaks have occurred. Forbes et al. (*2*) detected BOMV RNA in mouth swabs and internal parenchymal organs, except kidneys, of *M. condylurus* bats in Kenya in May 2018.

Most known outbreaks of EVD among humans were Zaire Ebola virus, including the large epidemic in West Africa during 2013–2016 (*3*). The reservoir hosts of Ebola virus (EBOV) remain unclear, but bats commonly are suspected. Viral RNA and EBOV antibodies have been detected in a few species of fruit bats (*4*,*5*). The discovery of BOMV supports the hypothesis regarding the role of bats as hosts of EBOVs, but further study is required to determine the bat species involved in viral transmission, prevalence of the virus in bat populations, and geographic distribution of the virus.

We detected BOMV RNA in free-tailed bats in N'Zerekore Prefecture, Guinea. We trapped bats in Guinea and Liberia during 2018–2019 (Table; Appendix, https://wwwnc.cdc.gov/EID/article/25/9/19-0581-App1.pdf) and detected BOM VRNA by reverse transcription PCR in 2 pools of kidney and lung samples from 2 *M. condylurus* bats captured in Yalenzou village in May 2018 (cycle threshold [C_t] 17.4 and 19.6) and in a pool of liver and spleen tissues (C_t 28.2) of an *M. condylurus* bat from a school in the city of N'Zerekore in March 2019 (Table). Blood, intestine, and brain samples were negative for viral RNA. Sequencing of the 483-bp fragment of the large gene (GenBank accession no. MK543447) demonstrated 99.3% identity with BOMV RNA from Sierra Leone (accession no. NC039345) and 98.3% identity with BOMV RNA from Kenya (accession no. MK340750).

Marí Saéz et al. (*5*) suggested that the Angolan free-tailed bat was the most plausible zoonotic source of the EVD

Table. Locations where free-tailed bats were trapped and tested for Bombali virus, Guinea and Liberia*

Location	Date	Species, no. tested (no. positive)			
		Total trapped	*Mops condylurus*	*Chaerephon pumilus*	*Chaerephon cf. major*
Yalenzou	2018 May 4	26	26 (2)	0	0
Gbao	2018 May 2	1	0	1	0
Yalenzou	2019 Mar 2	30	30	0	0
Bololowee†	2019 Mar 3	11	11	0	0
N'Zerekore, school	2019 Mar 5	47	27 (1)	0	20
N'Zerekore, house	2019 Mar 6	23	1	0	22
N'Zerekore, gazebo	2019 Mar 7	5	0	0	5
Dar Salam‡	2019 Mar 17	22	14	8	0
Total		165	109 (3)	9	47

*All bats were collected in N'Zerekore Prefecture, Guinea, except as indicated, and were tested by reverse transcription PCR for Bombali virus.
†Liberia.
‡Madina Oula Prefecture, Guinea.

epidemic in West Africa. In addition, EBOV nucleotide sequences previously have been found in *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata* bats in Gabon (*6*). He et al. (*7*) detected filovirus RNA in brown fruit bats (*Rousettus leschenaultii*) in China, and another study showed that 3 distinct groups of unclassified filoviruses are circulating in *Eonycteris spelaea* and *Rousettus* spp. fruit bats in China (*8*). These studies demonstrate that bats are promising targets for identifying emerging filoviruses, and additional Chiroptera species, both insectivorous and fruit bats, should be examined for EBOVs.

EBOV IgG was detected in the human population of Sierra Leone in 2006, 8 years before the EVD outbreak began in that country (*9*). Seroprevalence to EBOVs was also found in the medical staff of hospitals that were not involved in treating EVD-positive patients and in community contacts that worked with villages where EVD was not detected (*10*). The highest seroprevalence to EBOVs was found in the inhabitants of villages with the lowest number of documented EVD cases during the 2013–2016 outbreak in Sierra Leone (*10*). Cross-reactivity or nonspecific binding could be responsible for artifacts of immunoassay. However, other plausible explanations for the presence of antibodies against EBOV among persons with no symptoms of EVD exist, including subclinical EBOV infection in humans and antibody reactions to previously undiscovered, nonpathogenic filoviruses. The newly discovered BOMV could be a causative agent of these types of asymptomatic infections that produce antibodies with cross-reactivity to other EBOVs. Other undiscovered filoviruses also could be circulating in the region. Further surveillance with family-level primers is needed for insectivorous bats, as well as fruit bats and patients with acute infections.

Although BOMV had been detected in the northern part of Sierra Leone (*1*) and in the Taita Hills area of Kenya (*2*), we isolated it from bats in Guinea, far from these sites. Our finding provides additional evidence that BOMV is more widely distributed than previously suspected. Consequently, we advise screening of free-tailed bats for BOMV across their range. The high concentration of BOMV RNA we found in the internal organs of *M. condylurus* bats provides additional confirmation that BOMV could amplify in these bats and that this species is a reservoir host of this virus.

About the Author

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Appendix

Methods

Animal Sampling

We trapped bats in the villages of Yalenzou (7°42′29.99″ N, 8°41′20.60″ W) and Gbao (7°32′19.42″ N, 8°46′18.17″ W) in N'Zerekore Prefecture, Guinea in May 2018. In both villages, bats had roosted under the corrugated tin roofs of houses and granaries. We captured 26 free-tailed bats in Yalenzou and 1 bat in Gbao.

In March 2019, we conducted a second trapping session in N'Zerekore Prefecture. We captured 30 free-tailed bats in Yalenzou and 76 free-tailed bats in 3 different sites of N'Zerekore city (a school, a house, and a gazebo in the yard of another house). In addition, we trapped 11 Angolan free-tailed bats in Bololowee village in Liberia (7°41′19.21″ N, 8°40′32.47″ W), and 22 bats in Dar Salam village (9°58′36.88″ N, 12°20′21.08″ W) in Madina Oula Prefecture, Guinea.

We weighed; morphologically described; and measured the length of the head and body, forearm, tail, tibia, hind foot, and ear of trapped bats. Then we genotyped them by sequencing the cytochrome c oxidase subunit I (COI) gene using the following primers: ST-COI-F2 5′-CTCYACYAAWCAYAAAGACATTGGAAC-3′ (*1*) and jgHCO2198 5′-TAIACYTCIGGRTGICCRAARAAYCA-3′ (*2*).

We used cardiac puncture to collect blood into sterile tubes with 0.5 M EDTA. We then euthanized the animals and obtained sections of the brain, liver, spleen, kidney, lung, and intestines through sterile necropsy. In 2018, we collected a blood sample, a pool of lung and kidney tissues, and a pool of liver and spleen tissues from each animal. In 2019, we expanded the sample panel and separately collected the following into sterile tubes: blood; an oral swab; a

rectal swab; and lung, kidney, and intestine samples. Because previous reports did not find viral RNA in the liver or spleen (*3*,*4*), we homogenized these tissues in a single pool for each animal.

We stored blood fractions from N'Zerekore Prefecture at 10°C for 5–7 days, and at –28°C for the next 10 days before studying. We collected tissue samples in RNAlater (ThermoFisher Scientific, https://www.thermofisher.com) and stored them at 6–10°C for 12–15 days before studying. We stored samples from Madina Oula Prefecture at –70°C. We followed standard methods for the safe handling and sampling of small mammals potentially infected with infectious pathogens (*5*).

PCR Array and Sequencing Analyses

We extracted total RNA from blood, brain, and internal organs (lung, liver, spleen, kidney, and intestines) by using RIBO-prep kit (Central Research Institute of Epidemiology, Moscow, Russia, http://www.crie.ru). Then we prepared cDNA by using REVERTA-L (Central Research Institute of Epidemiology, Moscow, Russia). We screened samples for Bombali virus RNA by quantitative reverse transcription PCR by using Filo_UCD_qFor and Filo_UCD_qRev primers, and the Filo_UCD_probe probe sequence (*3*). We amplified cDNA with the Filo-MOD-FWD and FiloL.conR primers, and sequenced it with Filo-MOD-RVS probe (*3*).

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