Ebola Virus Neutralizing Antibodies in Dogs from Sierra Leone, 2017

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Ebola virus (EBOV) is a highly pathogenic zoonotic virus for which the reservoir host has not been identified. To study the role of dogs as potential hosts, we screened 300 serum samples from dogs in Sierra Leone and found EBOV neutralizing antibodies in 12, suggesting their susceptibility to natural infection.

Ebolaviruses (family *Filoviridae*) comprise highly pathogenic RNA viruses with zoonotic potential. After sporadic introduction from an animal reservoir into the human population, the main route of transmission has been from human to human, causing outbreaks of hemorrhagic fever with case-fatality rates up to 90% (*1*). Although molecular and serologic evidence strongly points toward certain species of bat as reservoir hosts for ebolaviruses (*2,3*), a bat-derived Ebola virus (EBOV) isolate has not yet been detected. Despite intensive serologic surveillance focusing on the role of bats, wildlife, and livestock in EBOV ecology (*2,4–7*), to our knowledge, only 2 reports describe analysis of serum from dogs in Gabon and Liberia after Ebola virus disease (EVD) outbreaks in 2001 (Gabon) and 2014–2016 (Liberia) (*8,9*). Although antibodies against EBOV were detected by indirect ELISA, neither EBOV antigen nor viral genome was detected in samples from Gabon. The highest seroprevalence (31.8%) was reported from villages where dogs were reportedly exposed to the virus through contact with human EVD patients or by eating infected animal carcasses (*8*). In Liberia, a multiplex approach indicated that 47 (73%) of 64 dogs had potentially been exposed to filoviruses (*9*). To further investigate the role of dogs in EBOV ecology, we collected 300 serum samples from 174 male (58%) and 126 female (42%) dogs in Moyamba District, Sierra Leone (Figure 1).

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The Study

We sampled only owned and healthy dogs from communities that have been affected by the West Africa EVD outbreak (Appendix, https://wwwnc.cdc.gov/EID/article/26/4/19-0802-App1.pdf). Sampling was performed in rural and urban areas that had been affected by the historically largest EVD outbreak involving the Makona strain during 2014–2016 in West Africa. At the time of sample collection (October–December 2017), of the 300 dogs, 163 were >2 years of age (Table 1). Animals were handled according to a Njala University Institutional Review Board protocol (no. IRB00008861/FWA00018924).

Initially, we screened dog serum samples for the presence of EBOV nucleoprotein (NP)–specific antibodies in an indirect ELISA, as previously described for pigs (*5*), with slight modifications. Using a horseradish peroxidase–labeled protein A/G–specific conjugate, we considered 36 (12%) serum samples to be reactive toward the *Escherichia coli*–derived EBOV-NP (Tables 1, 2). Subsequent Western blot analyses based on insect cell–derived EBOV-NP (*5*) confirmed the presence of EBOV-NP reactive antibodies in 20 (6.6%) samples. Furthermore, we performed virus neutralization tests (VNTs) by using transcription and replication competent virus-like particles (trVLP) and authentic EBOV (variant Mayinga) as described previously (*5,10*) (Appendix). We found that 12 (4%) serum samples efficiently inhibited EBOV infection with robust neutralizing titers of 1:16–1:45 and that another 6 samples had weakly positive titers of 1:11–1:13 (Tables 1, 2). Overall, titers from the trVLP-based VNTs with an established cutoff at 80% inhibition of reporter activity were comparable to those of VNTs with live virus (Figure 2).

Conclusions

EBOV seroprevalence detected by ELISA in dogs from EVD-endemic areas in Gabon (25.2%–31.8%) (*8*) was lower than that detected in dogs in our study in Sierra Leone (12%). Technically, differences in detectable seroprevalence could be explained by different preparations used (virus-infected cell lysates in ELISA in
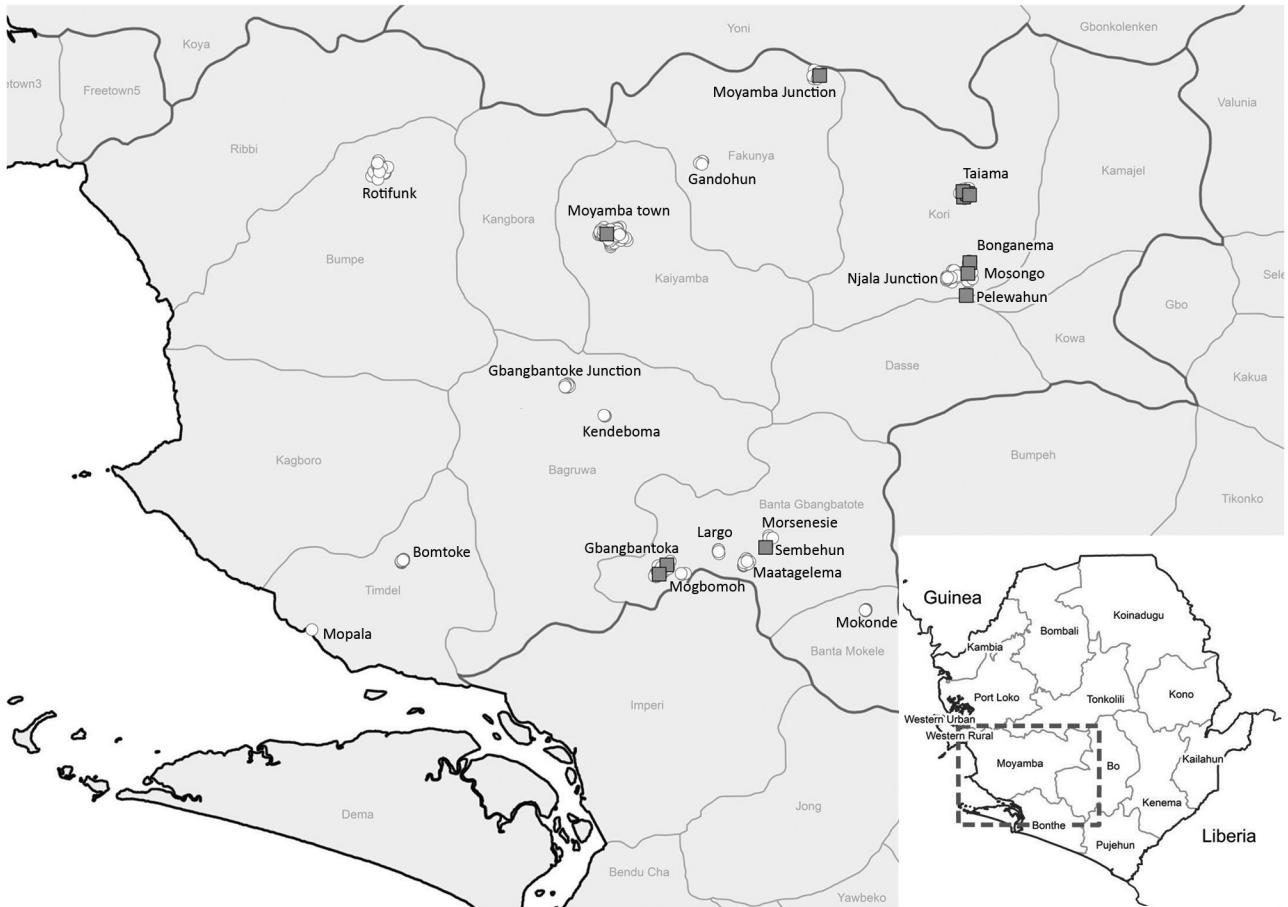
Figure 1. Sampling locations for study of Ebola virus neutralizing antibodies in dogs, Moyamba District, Sierra Leone, 2017. White circles indicate sampling locations; gray squares indicate dog serum samples with virus neutralizing activity. Inset shows location of Moyamba district in Sierra Leone.

Gabon [8] compared with single EBOV-NP preparations in our study). Apart from that difference, the observed variation might depend on selected sampling areas, animals, and time points of sampling because stability and persistence of neutralizing and NP-reactive antibodies in dogs after exposure are unknown. In pigs experimentally infected with EBOV, NP-specific antibody titers decreased within 28 days after infection, but neutralizing antibodies seemed to persist longer (11). Of note, Marburg virus IgG in convalescent *Rousettus aegyptiacus* bats decreased to undetectable titers at 3 weeks after infection (12). Nonetheless, the recent report of EBOV neutralizing antibodies in human survivors up to 40 years after infection (13) suggests a rather long-lasting but host-dependent antibody response after infection.

Reactivity of dog serum to EBOV-NP in ELISA and Western blots suggests exposure of the dogs to antigenically related ebolaviruses or Ebola-like viruses, as previously described for pigs (5). In our study, a novel ebolavirus, referred to as Bombali virus, which was recently discovered in insectivorous bats from the Bombali

Table 1. EBOV-specific antibodies detected in dog serum samples, by dog age, collected in Moyamba District, Sierra Leone, October–December 2017*

Age, mo.	No. samples tested	EBOV-NP ELISA, no. (%) reactive	Confirmatory EBOV-NP WB, no. (%) reactive	EBOV VNT	
				No. (%) positive	Titers
<12	27	2 (7.4)	0	0	NA
12 –18	60	7 (11.7)	6 (10.0)	5 (8.3)	1:11, 1:11, 1:13, 1:16, 1:27
19–24	50	4 (8.0)	3 (6.0)	1 (2.0)	1:11
25–36	90	11 (12.2)	5 (5.6)	4 (4.4)	1:16, 1:16, 1:19, 1:32
37–48	39	7 (17.9)	5 (12.8)	5 (12.8)	1:11, 1:16, 1:19, 1:23,1:45
>48	34	5 (14.7)	1 (2.9)	3 (8.8)	1:11, 1:19, 1:19
Total	300	36 (12.0)	20 (6.7)	18 (6.0)	

*EBOV, Ebola virus; NA, not applicable; NP, nucleoprotein; VNT, virus neutralization test; WB, Western blot.

Table 2: EBOV-specific antibodies detected in dog serum samples, according to sampling region, Sierra Leone, October–December 2017*

Region	No. samples tested	EBOV-NP ELISA, no. (%) reactive	Confirmatory EBOV-NP WB, no. (%) reactive	EBOV VNT No. (%) positive	Titers
Bomtoke	11	0	0	0	NA
Bonganema	8	3 (37.5)	3 (37.5)	1 (12.5)	1:16
Gandohun	4	0	0	0	NA
Gbangbantoke	24	3 (12.5)	0	3 (12.5)	1:13, 1:19, 1:19
Gbangbantoke Junction	14	0	0	0	NA
Kendeboma	7	0	0	0	NA
Largo	9	0	0	0	NA
Matagelema	16	1 (6.3)	1 (6.3)	0	NA
Mogbomoh	4	0	0	0	NA
Mokonde	14	1 (7.1)	1 (7.1)	0	NA
Mopala	1	0	0	0	NA
Morsenesie	4	2 (50.0)	0	0	NA
Mosongo	26	5 (19.2)	3 (11.5)	2 (7.6)	1:11; 1:16
Moyamba Junction	16	2 (12.5)	2 (12.5)	1 (6.2)	1:23
Moyamba Town	62	1 (1.6)	1 (1.6)	1 (1.6)	1:32
Njala Junction	15	2 (13.3)	1 (6.6)	1 (6.6)	1:11
Pelewahun	14	2 (14.3)	2 (14.3)	1 (7.1)	1:45
Rotifunk	21	2 (9.5)	2 (9.5)	2 (9.5)	1:11, 1:11
Sembehun	7	3 (42.9)	1 (14.3)	1 (14.2)	1:27
Taiama	23	9 (39.1)	3 (13.0)	5 (21.7)	1:11, 1:16, 1.16, 1:19, 1.19
Total	300	36 (12.0)	20 (6.7)	18 (6.0)	

*EBOV, Ebola virus; NA, not applicable; NP, nucleoprotein; VNT, virus neutralization test; WB, Western blot.

District in Sierra Leone (*3*), may account for cross-reactivity of the dog serum to EBOV-NP. The virus neutralization induced by specific binding to the EBOV surface glycoprotein suggests exposure of the dogs to EBOV or to a closely related ebolavirus eliciting cross-neutralizing antibodies. Although in vitro assays using an EBOV glycoprotein-pseudotyped virus revealed that infectivity is restricted in canine cells (*14*), detection of EBOV (cross-)neutralizing antibodies in dogs supports susceptibility to natural EBOV or ebolavirus infection.

The dog with the highest neutralizing titer (1:45) was 48 months of age; other dogs with neutralizing antibodies were 28–72 months of age at the time of blood collection, suggesting exposure during the West Africa EVD outbreak. However, information on past clinical signs in the dogs was not recorded, and the route of exposure or potential infection remains unknown. Exposure of dogs during the EVD outbreak in Gabon was assumed to result from consuming virus-infected carcasses or licking vomitus from EVD patients (*8*). Samples from those dogs, which displayed no clinical signs, tested negative for EBOV RNA (*8*). Furthermore, recent testing of 240 swab samples from dogs from Bombali District revealed no detectable filovirus RNA in the specimens; serologic assays were not performed (*3*).

Although most seropositive dogs in our study were potentially exposed to the virus during the EVD epidemic, 2 dogs with neutralizing antibodies (titers 1:16 and 1:27) were only 16 and 18 months of age, indicating contact with ebolavirus after the World Health Organization officially declared the end of the EVD outbreak in Sierra Leone by mid-March 2016 (*15*). Of note, some of the seropositive dog samples from Gabon were collected from areas without reported human EVD cases (*8*). These findings suggest exposure and immunogenic stimulation of free-ranging dogs by a source other than secretions from acutely
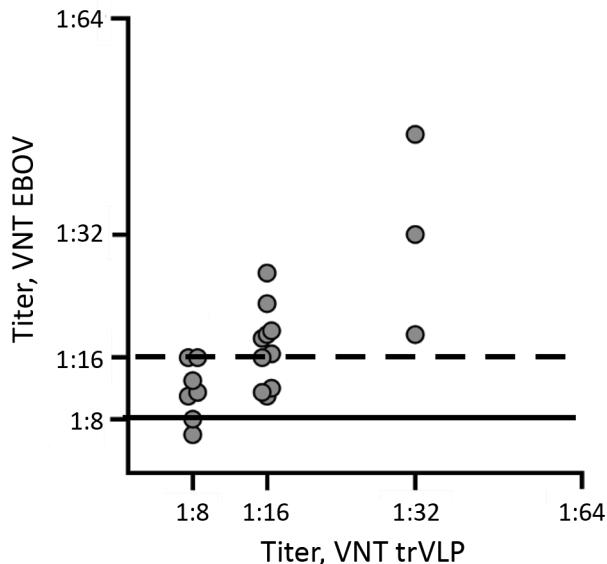

Figure 2. Analysis of dog serum samples (circles) in VNTs for study of EBOV neutralizing antibodies in dogs, Moyamba District, Sierra Leone, 2017. Comparison of dog serum titers obtained in VNTs was based on live EBOV (variant Mayinga) and EBOV trVLP. For VNT using authentic EBOV, serum samples with a titer ≤1:8 (horizontal solid line) are counted as negative; samples with a neutralizing titer >1:8 are considered positive. For trVLP-based VNT, titers equal to 1:16 (horizontal dashed line) are counted as positive. EBOV, Ebola virus; trVLP, transcription and replication competent virus-like particles; VNTs, virus neutralization tests.

infected patients or infection with a heterologous ebolavirus circulating in wildlife reservoir hosts.

To date, neither evidence of clinical EVD in dogs nor virus shedding with subsequent transmission to humans has been reported. However, whether dogs play an active role in EBOV ecology, represent dead-end hosts, or act as passive virus carriers mechanically spreading the virus after licking and feeding on infected carcasses or fomites remains unknown. Therefore, organ tissues (including salivary glands, bladder, and intestines) or secretions that might lead to virus shedding and transmission should be collected from dogs during any future EVD epidemic.

This report of EBOV neutralizing antibodies in dogs suggests their susceptibility to natural infection by EBOV or antigenically related ebolaviruses. Considering the abundance of dogs and their close association with humans in Africa, the comparably low number of human EVD outbreaks in the past most likely indicates that dogs do not represent a reservoir or intermediate host for EBOV.

Acknowledgements

About the Author

Dr. Fischer is a postdoctoral fellow at the Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Germany. Her research focuses on highly pathogenic ebolaviruses and henipaviruses, their virus–host interactions, and the epidemiologic role of domestic animals and livestock in their ecology.

References

1. Feldmann H, Geisbert TW. Ebola haemorrhagic fever. Lancet. 2011;377:849–62. https://doi.org/10.1016/S0140-6736(10)60667-8
2. Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature. 2005;438:575–6. https://doi.org/10.1038/438575a
3. Goldstein T, Anthony SJ, Gbakima A, Bird BH, Bangura J, Tremeau-Bravard A, et al. The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. Nat Microbiol. 2018;3:1084–9. https://doi.org/10.1038/s41564-018-0227-2
4. De Nys HM, Kingebeni PM, Keita AK, Butel C, Thaurignac G, Villabona-Arenas CJ, et al. Survey of Ebola viruses in frugivorous and insectivorous bats in Guinea, Cameroon, and the Democratic Republic of the Congo, 2015–2017. Emerg Infect Dis. 2018;24:2228–40. https://doi.org/10.3201/eid2412.180740
5. Fischer K, Jabaty J, Suluku R, Strecker T, Groseth A, Fehling SK, et al. Serological evidence for the circulation of ebolaviruses in pigs from Sierra Leone. J Infect Dis. 2018 218(suppl_5):S305–11.
6. Leroy EM, Rouquet P, Formenty P, Souquière S, Kilbourne A, Froment JM, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science. 2004;303:387–90. https://doi.org/10.1126/science.1092528
7. Weingartl HM, Nfon C, Kobinger G. Review of Ebola virus infections in domestic animals. Dev Biol (Basel). 2013;135:211–8. https://doi.org/10.1159/000178495
8. Allela L, Boury O, Pouillot R, Délicat A, Yaba P, Kumulungui B, et al. Ebola virus antibody prevalence in dogs and human risk. Emerg Infect Dis. 2005;11:385–90. https://doi.org/10.3201/eid1103.040981
9. Haun BK, Kamara V, Dweh AS, Garalde-Machida K, Forkay SSE, Takaaze M, et al. Serological evidence of Ebola virus exposure in dogs from affected communities in Liberia: a preliminary report. PLoS Negl Trop Dis. 2019;13:e0007614. https://doi.org/10.1371/journal.pntd.0007614
10. Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. Phase 1 trials of rVSV Ebola vaccine in Africa and Europe. N Engl J Med. 2016;374:1647–60. https://doi.org/10.1056/NEJMoa1502924
11. Pickering BS, Collignon B, Smith G, Marszal P, Kobinger G, Weingartl HM. Detection of Zaire ebolavirus in swine: assay development and optimization. Transbound Emerg Dis. 2017.
12. Schuh AJ, Amman BR, Sealy TK, Kainulainen MH, Chakrabarti AK, Guerrero LW, et al. Antibody-mediated virus neutralization is not a universal mechanism of Marburg, Ebola or Sosuga virus clearance in *Egyptian rousette* bats. J Infect Dis. 2018;219:1716–21.
13. Rimoin AW, Lu K, Bramble MS, Steffen I, Doshi RH, Hoff NA, et al. Ebola virus neutralizing antibodies detectable in survivors of the Yambuku, Zaire outbreak 40 years after infection. J Infect Dis. 2018;217:223–31. https://doi.org/10.1093/infdis/jix584
14. Han Z, Bart SM, Ruthel G, Vande Burgt NH, Haines KM, Volk SW, et al. Ebola virus mediated infectivity is restricted in canine and feline cells. Vet Microbiol. 2016;182:102–7. https://doi.org/10.1016/j.vetmic.2015.11.011
15. World Health Organization. WHO statement on end of the Ebola flare-up in Sierra Leone [cited 2019 Jan 19]. https://www.afro.who.int/media-centre/statements-commentaries/who-statement-end-ebola-flare-sierra-leone

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Appendix

Methods

Animal sampling

We sampled 300 owned and apparently healthy dogs from Moyamba district from different communities (Figure 1, Table 2) between September and November 2017. Communities such as Moyamba Junction and Taiama have been known for their history of EVD patients during the EVD epidemic (2014-2016). Dogs are free ranging within the communities. Owners do not feed their dogs. Thus, dogs scavenge for food within the communities. Alternatively, dogs go hunting for food in the nearby forests. During the EVD outbreak, unsafe burials were performed allowing the dogs to come into contact with these bodies as a possible source of infection.

Most of the sampling areas / communities are close to major bat caves. Therefore, dogs may be exposed to bats directly or indirectly.

Control serum samples

For the establishment of the dog ELISA, we used 72 sera collected previously from German dogs as negative controls. These samples were also used to determine the cut-off by calculating the mean plus 3 standard deviations. None of the German sera ranged above that cut-off. Defined positive controls were unfortunately not available prior to our testing. Thus, we used sera from VLP immunized pigs as a positive control in ELISA. For Western Blot, His-tag specific monoclonal antibodies were used to detect the antigen.

Indirect IgG ELISA based on *E. coli* derived EBOV NP

The ELISA was performed as described (*1*) with the modification of using a horseradish peroxidase-labelled Protein A/G-specific conjugate.

Virus neutralization test (VNT) using authentic EBOV

Sera were incubated at 56°C for 30 min for complement inactivation. Then, sera were serially diluted and 100 $TCID_{50}$ units of Ebola virus (Zaire, isolate Mayinga, AF086833) were added to each serum dilution. Following incubation at 37°C for 1 hour, Vero cell suspension in DMEM containing 2% fetal calf serum was added. Plates were then incubated at 37°C with 5% CO_2 and cytopathic effects (CPE) were analysed at 7 dpi. Neutralization was defined as reduction of CPE in serum dilutions compared to positive controls. For each sample, we performed four replicates. Neutralization titres were calculated as geometric mean titres (GMT) of these four replicates. We defined a sample negative if the serum sample did not exhibit any neutralization effect at a 1:8 dilution (the lowest dilution tested). A corresponding serum control was assayed for each sample to exclude any potential cytotoxic effects. As a positive control for neutralization, we routinely use a purified ovine anti EBOV IgG product that exhibits high neutralization titers (EBOTab) (*2*). Further controls included virus-infected cells without the addition of antisera to determine the cytopathic effect as well as non-infected cells as Mock control. All these controls performed as expected.

In addition to analysis of ELISA reactive dog sera in VNT, we tested a number of ELISA negative dog serum samples that turned out to be non-neutralizing and not toxic to the cell monolayers.

Further information concerning the trVLP-based VNT are mentioned elsewhere (*1*).

Limitations of the study

To our knowledge, no study has previously reported EBOV neutralizing antibodies in dogs from Sierra Leone. Nonetheless, there are some limitations of this study. The development of assays for dog surveillance is a strength of this study, however, a defined positive control serum panel is lacking. Further, in this study, we only test for IgG antibodies. Expanding future testing for IgM antibodies as well as testing for viral genome presence would broaden our knowledge of the role of dogs in the ebolavirus transmission chain. Another limitation is that the age of the animals is reported according to the dog owner which may not be absolutely accurate.

References

1. Fischer K, Jabaty J, Suluku R, Strecker T, Groseth A, Fehling SK, et al. Serological Evidence for the circulation of Ebolaviruses in pigs From Sierra Leone. J Infect Dis. 2018 218(suppl_5):S305–S11.

2. Dowall SD, Callan J, Zeltina A, Al-Abdulla I, Strecker T, Fehling SK, et al. Development of a cost-effective ovine polyclonal antibody-based product, EBOTAb, to treat ebola virus infection. J Infect Dis. 2016;213:1124–33. PubMed https://doi.org/10.1093/infdis/jiv565