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Exposure to Lyssaviruses in Bats of the Democratic Republic of the Congo

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ABSTRACT: Lyssavirus infections in the Democratic Republic of Congo are poorly documented. We examined 218 bats. No lyssavirus antigens were detected but *Lagos bat virus* (LBV) neutralizing antibodies (VNA) were detected in *Eidolon helvum* and *Myonycteris torquata*. Four samples with LBV VNA reacted against *Shimoni bat virus*.

Rabies is a disease caused by viruses of the genus *Lyssavirus*. Six lyssaviruses have been isolated from animal samples originating in Africa but only three are suspected to naturally circulate in bats; *Lagos bat virus* (LBV), *Duvenhage virus* (DUVV), and *Shimoni bat virus* (SHIBV). Neutralizing antibodies against *West Caucasian bat virus* (WCBV) have been detected in sera of bats from Kenya (Kuzmin et al. 2008a), although the virus was originally isolated from an insectivorous bat in western Russia (Botvinkin et al. 2003). Canine rabies virus is common in all provinces of the Democratic Republic of the Congo (DRC) including the densely populated Kinshasa Province. Clinical rabies cases were observed within Kinshasa from 20 municipalities, with 128 cases from dogs (*Canis lupus familiaris*; *n*=119) and cats (*Felis catus*; *n*=9), in 2009–13 (Twabela et al. 2016). Recently, bats have emerged as an important reservoir of lyssaviruses in various regions of the world (Rupprecht et al. 2011). In the DRC, there are no reliable data on potential wildlife reservoirs of lyssaviruses, which thus precludes measurement of the impact of these viruses on human and animal health. We hypothesized that there would be evidence of lyssavirus circulation in DRC bats, as reported elsewhere in Africa, and herein we describe preliminary efforts to address the knowledge gap about lyssavirus circulation in DRC bats.

We captured 218 bats representing 18 species at collection sites (Kinshasa Province, Bas-Congo Province, and near Kisangani in Orientale Province) during June 2011–July 2012. Captured bats were identified based on external morphology using field guides (Kingdon 2010). Following capture, animals were anesthetized by intramuscular injection of ketamine hydrochloride (0.05–0.1 mg/g body weight). Bats were euthanized by exsanguination or cervical dislocation under anesthesia. Blood was collected via cardiac puncture and serum was separated by centrifugation. Bats were then subjected to full necropsy and brain tissues were collected. Samples were stored in liquid nitrogen in the field and then at –80 C until analysis.

Bat brains (*n*=218) were screened for lyssavirus antigen by the direct fluorescent antibody test as described by Dean et al. (1996) using monoclonal (Fujirebio Diagnostics Inc., Malvern, Pennsylvania, USA) or polyclonal (Chemicon Int., Temecula, California, USA) fluorescein isothiocyanate-labeled anti-rabies virus antibodies.

Virus-neutralizing antibodies (VNA) in bat sera were detected by a modification of the rapid fluorescent focus inhibition test as described by Kuzmin et al. (2008b). Sera were tested against LBV (AFR1999, lineage A; *n*=210), DUVV (South African isolate from 1970, lineage A; *n*=216), SHIBV (*n*=205), and WCBV (*n*=211). If a reduction or absence of fluorescence was observed, the serum sample

TABLE 1. Sera from bats collected in the Democratic Republic of the Congo, 2011–12, and tested for lyssavirus neutralizing antibodies (no. positive/no. tested). Dashes indicate insufficient sample for analysis.

Species	LBV[a]	DUVV[b]	SHIBV[c]	WCBV[d]
Chaerephon pumilus	0/23	0/23	0/24	0/24
Chaerephon sp.	0/20	0/22	0/20	0/20
Eidolon helvum	6/18	0/19	4/19	0/19
Glauconycteris argentata	0/1	0/1	—	0/1
Hipposideros fuliginosus	0/20	0/20	0/20	0/20
Hipposideros gigas	0/1	0/1	0/1	0/1
Hypsignathus monstrosus	0/2	0/2	0/2	0/2
Megaglossus woermanni	0/8	0/8	0/8	0/8
Micropteropus pussilus	0/1	0/1	0/1	0/1
Mimetillus moloneyi	0/1	—	—	—
Miniopterus sp.	0/38	0/39	0/36	0/38
Mops condylurus	0/31	0/33	0/30	0/30
Myonycteris torquata	2/6	0/6	0/5	0/6
Myotis sp.	0/1	0/1	0/1	0/1
Neoromicia sp.	0/1	0/1	0/1	0/1
Pipistrellus sp.	0/35	0/36	0/34	0/36
Rhinolophus sp.	0/1	0/1	0/1	0/1
Scotophilus dinganii	0/2	0/2	0/2	0/2
Total	8/210	0/216	4/205	0/211

[a] *Lagos bat virus*.
[b] *Duvenhage virus*.
[c] *Shimoni bat virus*.
[d] *West Caucasian bat virus*.

was subjected to additional titration in dilutions of 1:10 to 1:1,250. The 50% end-point neutralizing titers were calculated following Reed and Muench (1938). Only samples with a 50% end-point neutralizing titer >1 $\log_{10}$ (e.g., fewer than five fields contained infected cells at a serum dilution of 1:10) were considered positive. Cytotoxic and positive samples were tested at least twice prior to reporting a final result.

All bats appeared to be healthy upon capture and no lyssavirus antigens were detected in brain impressions of any bats. We detected LBV VNA in sera from two little collared fruit bats (*Myonycteris torquata*) and six African straw-colored fruit bats (*Eidolon helvum*). None of the 131 insectivorous and nectarivorous bats had serologic evidence of exposure to LBV. Four *E. helvum* serum samples with LBV VNA also showed evidence of SHIBV VNA. Virus-neutralizing antibodies against DUVV and WCBV were not detected in any sera (Table 1). Among the four *E. helvum* sera with reactivity against LBV and SHIBV, three had a higher titer against LBV and one had equal titers against both viruses (Table 2).

TABLE 2. Titers of antibody-positive bat sera collected in the Democratic Republic of the Congo, 2011–12. All bats positive for antibody to *Lagos bat virus* (LBV) and *Shimoni bat virus* (SHIBV) were captured at the Kisangani location and titers are presented on a $\log_{10}$ scale. M = male; F = female.

Species	Sex	LBV titer	SHIBV titer
Eidolon helvum	M	1.47	0
E. helvum	M	1.47	1.26
E. helvum	F	3.41	1.2
E. helvum	M	1.9	0
E. helvum	F	2.26	2.1
E. helvum	F	2.71	2.71
Myonycteris torquata	F	1.35	0
M. torquata	F	1.5	0

Bats are reservoirs of many viruses (Kuzmin et al. 2011a). Although no lyssaviruses were isolated from bats we collected, serologic data suggest prior exposure of *E. helvum* and *M. torquata* fruit bats to LBV and potential cross-reactivity of some LBV-positive *E. helvum* sera to SHIBV. *Lagos bat virus* has been isolated from several bat species across the African continent (Markotter et al. 2008). Suggested reservoir species of LBV include *E. helvum*, Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*), and Egyptian rousettes (*Rousettus aegyptiacus*) (Markotter et al. 2008). *Shimoni bat virus* has only been isolated from an insectivorous bat, the striped leaf-nosed bat (*Hipposideros vittatus*) in Kenya, although evidence of SHIBV VNA has been detected, and cross-reactivity against LBV and SHIBV observed, in several bat

species including *E. helvum* (Kuzmin et al. 2011b). Considering our results, it is evident that LBV is the most documented bat lyssavirus from studies in Africa, and *E. helvum* is likely to be a natural reservoir of LBV in DRC.

We also detected LBV VNA in a species (*M. torquata*) in which it had not previously been observed. It is unclear whether exposure to LBV may have resulted from spillover from a reservoir, or whether this species plays a role in natural circulation of LBV, or whether the exposure was potentially associated with an undiscovered or unrecognized lyssavirus. Both *M. torquata* and *E. helvum* are frugivorous bats with a preference for fruits of the fig genus (*Ficus*; Kingdon et al. 2013). The LBV antibody-positive bats of these two species were identified within 4 km of each other. While it is impossible to determine if cross-species transmission occurred between these species, or whether the LBV VNA detected in *M. torquata* definitively represents exposure to LBV, the feeding habits and proximity of capture locations for these bats suggest that interspecies interactions associated with foraging may play a role in LBV transmission.

In the DRC, data are lacking about potential wildlife reservoirs of lyssaviruses. Poor surveillance of wildlife reservoirs for lyssaviruses also exists in most other African countries, and novel lyssaviruses continue to be isolated from African wildlife. Enhanced surveillance targeting diverse species of bats would improve the epidemiologic understanding of lyssavirus circulation among African bats.

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