Bartonella Species in Bats (Chiroptera) and Bat Flies (Nycteribiidae) from Nigeria, West Africa

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Abstract

Previous and ongoing studies have incriminated bats as reservoirs of several emerging and re-emerging zoonoses. Most of these studies, however, have focused on viral agents and neglected important bacterial pathogens. To date, there has been no report investigating the prevalence of *Bartonella* spp. in bats and bat flies from Nigeria, despite the fact that bats are used as food and for cultural ritual purposes by some ethnic groups in Nigeria. To elucidate the role of bats as reservoirs of bartonellae, we screened by molecular methods 148 bats and 34 bat flies, Diptera:Hippoboscoidea:Nycteribiidae (*Cyclopodia greeffi*) from Nigeria for *Bartonella* spp. Overall, *Bartonella* spp. DNA was detected in 76 out of 148 (51.4%) bat blood samples tested and 10 out of 24 (41.7%) bat flies tested by qPCR targeting the 16S–23S internal transcribed spacer (ITS) locus. *Bartonella* was isolated from 23 of 148 (15.5%) bat blood samples, and the isolates were genetically characterized. Prevalence of *Bartonella* spp. culture-positive samples ranged from 0% to 45.5% among five bat species. *Micropteropus* spp. bats had a significantly higher relative risk of 3.45 for being culture positive compared to *Eidolon helvum*, *Epomophorus* spp., *Rhinolophus* spp., and *Chaerephon nigeriae*. *Bartonella* spp. detected in this study fall into three distinct clusters along with other *Bartonella* spp. isolated from bats and bat flies from Kenya and Ghana, respectively. The isolation of *Bartonella* spp. in 10.0–45.5% of four out of five bat species screened in this study indicates a widespread infection in bat population in Nigeria. Further investigation is warranted to determine the role of these bacteria as a cause of human and animal diseases in Nigeria.

Key Words: *Bartonella*—Bats—Vector—Reservoir host—Flies.

Introduction

THE ORDER CHIROPTERA IS THE MOST diverse and geographically distributed mammalian taxon found on all continents except Antarctica (Schipper et al. 2008). They are the second-most speciose group of mammals after Rodentia (Simmons 2005). In Nigeria, bats form an important part of cultural and traditional ceremonies among some ethnic groups and serve as food and a protein source (Billeter et al. 2012). Bats are unique among mammals in their capability to fly and cover long distances during seasonal migrations and in their potential for living in diverse colonial populations. These characteristics together with the long life span of some species and the ability to inhabit a multitude of diverse eco-

logical niches make bats among the most successful species adapting and surviving in different climates on earth. As a result, there is a renewed global interest in bats as potential reservoir hosts and vectors of zoonotic pathogens (Calisher et al. 2006, Wong et al. 2007, Kuzmin et al. 2011, Wang et al. 2011).

Multiple studies have highlighted the potential role of bats as natural reservoirs for numerous pathogens including viruses and bacteria (Hanson 1970, Messenger et al. 2003, Loftis et al. 2005, Matthias et al. 2005, Gibson et al. 2005, Calisher et al. 2006, Reeves et al. 2007, Evans et al. 2009, Bai et al. 2011, Lin et al. 2012). Research studies have confirmed the presence of *Bartonella* spp. in bats from Peru, Guatemala, Kenya, and the United Kingdom (Concannon et al. 2005,

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Kosoy et al. 2010, Bai et al. 2011, 2012), and in bat ectoparasites from Egypt, the United States, and Ghana (Loftis et al. 2005, Reeves et al. 2005, 2007, Billetter et al. 2012). However, knowledge on bat bartonellae is preliminary and further research is warranted. Moreover, no study on *Bartonella* spp. in bats and bat flies from Nigeria has been carried out to date. The objectives of this study were therefore to: (1) Screen bats and bat flies from Nigeria for bartonellae, and determine the prevalence of *Bartonella* spp. in bat populations from Nigeria and (2) genetically characterize bat-associated *Bartonella* spp. from Nigeria and evaluate their genetic diversity.

Materials and Methods

Ethics statement

This study and sampling protocol were approved by the Institutional (National Veterinary Research Institute [NVRI], Vom Nigeria), Animal Care and Use Committee (approval number AEC/03/01/13). Oral permission to place hand nets in the study area was granted by village heads and residents. Animals were treated in a humane manner and in accordance with authorizations and guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research of the American Psychological Association (APA) for use by scientists working with nonhuman animals (American Psychological Association Committee on Animal Research and Ethics) in 2010.

Study area

Blood and ectoparasites from bats were collected from Bauchi (10°18'57"N, 09°50'39"E) and Gboko (7°19'30"N, 9°0'18"E) towns, Nigeria (Fig. 1). Sampling sites were chosen on the basis of available information on bat roosts and foraging sites.

Samples collection

Bats were collected by hand or mist nets around roosts or in locations of nocturnal foraging. In each sampling site, six mist nets (9×3 meters each) were set up for 18–24 h, and

checked every hour. Both adult and subadult bats were randomly selected; however, preference was given to adults. Trapped bats were collected and inspected for the presence of ectoparasites. When present, the ectoparasites from each bat were collected and placed in labeled vials containing 70% ethanol.

The bats were then morphologically identified to genus and when possible to species level (Bergmans 1988) by field experts (Gablong P. Litwat, NVRI Outstation Laboratory, Bauchi, Nigeria, and Nyor Gelahan, College of Agriculture, Yandev, Nigeria) and exsanguinated by cardiac puncture following anesthesia using isoflurane gas. About 0.5–2 mL of blood (depending on the bat size) was collected from each bat into EDTA tubes and labeled. Blood samples were transported from the field in cool boxes to the Parasitology Laboratory, NVRI, Vom, and stored at –20°C until shipped to the Koret School of Veterinary Medicine in Israel for further analysis.

Identification of bat flies

Initial examination of each specimen was done at 10× and 30× with a Meiji stereo microscope with the specimen in an excavated glass block and 70% ethanol and quickly keyed using Theodor (1967) to the genus *Cyclopodia*. Further detailed examination was performed at 60× of the chaetotaxy (bristles) of the female abdomen. Females are distinctive and are easy to identify. Males, however, are difficult, needing very careful examination of male claspers, which on some specimens, needed to be levered gently out of the male abdomen. All materials were further compared to the *Cyclopodia* specimens in the entomology collection of the Natural History Museum, London, which confirmed the initial identification of *C. greeffi*.

Bartonella spp. culture from bat blood

Fifty microliters of thawed EDTA–blood was plated onto chocolate agar and sealed with a shrink seal. The plates were incubated at 35°C and 5% carbon dioxide. Bacterial growth was monitored at the end of the first week and then on alternate days for up to 45 days. Bacterial colonies were

FIG. 1. Map of Nigeria, West Africa showing the two sampling sites, Bauchi and Gboko, and the number (*n*) of bats sampled at each site. Color images available online at www.liebertpub.com/vbz

presumptively identified as *Bartonella* spp. based on their morphology. Suspected *Bartonella* colonies were randomly selected from the original agar plate, streaked onto secondary chocolate agar plates, and incubated at the same conditions until sufficient growth was observed. Pure cultures were harvested and stored in 10% glycerol at -80°C .

DNA extraction from *Bartonella* cultures

A heavy suspension of the microorganisms was heated for 10 min at 95°C followed by 1 min of centrifugation at 8000 rpm to precipitate the lysed bacteria. The supernatant containing the genomic DNA to be used as DNA template in the PCR amplification assay was then pipetted into a clean 1.5-mL centrifuge tube.

DNA extraction from bat flies

Prior to DNA extraction, the bat flies were removed from the ethanol vials and rinsed in sterile phosphate-buffered saline (PBS). Bat flies were treated individually, except in cases where more than one fly was identified on a host (five bats), and then treated as a pool of two to four flies. The flies were first triturated using a sterile scalpel blade and manually homogenized using a plastic pestle. DNA extraction was performed using a DNeasy blood and tissue kit (QIAGEN, GmbH Germany) according to the manufacturer's instructions.

DNA extraction from blood of bats

DNA from bats' blood was extracted using the DNeasy blood and tissue kit (QIAGEN, GmbH Germany) according to the manufacturer's instructions. DNA quality and concentrations were assessed with a NanoVue spectrometer (GE Healthcare, UK Limited).

Real-time PCR amplification of the *rpoB* gene fragment and 16S–23S internal transcribed spacer locus

Initial detection of *Bartonella* spp. was performed using real-time PCR analysis targeting the *rpoB* and 16S–23S internal transcribed spacer (ITS) region in genomic DNA extracted directly from blood of bats and bat flies using oligonucleotide primers listed in Table 1. Real-time PCR was carried out using the StepOne (Applied Biosystems) real-time system under the following conditions, one cycle of

95°C for 15 min followed by 50 cycles of 15 s at 95°C , 30 s at 57°C , and 10 s at 76°C with data collection in the FAM channel. The PCR was performed in 20- μL reaction volumes containing 3 μL of DNA, 1 μL of 10 mM of each primer, 10 μL Absolute Blue dye (Thermo Scientific, Surrey, UK), and 5 μL ultrapure PCR water (Thermo Scientific, Surrey, UK). *Bartonella henselae* DNA extracted from blood of a naturally infected cat was used as a positive control, and DNA from a cat negative by PCR for *Bartonella* spp. was used as a negative control. One sample containing all the ingredients of the reaction except DNA was used as a non-template control (NTC). All of the controls were included in each PCR reaction to evaluate the presence of appropriate melting curves and possible contamination.

Conventional PCR amplification of the *gltA* and *rpoB* fragments

PCR amplifications were performed using oligonucleotide primers (Table 1) to amplify a 379-bp fragment of the *gltA* and a 830-bp fragment of the *rpoB* genes of *Bartonella* spp. under the cycling conditions, as previously described (Norman et al. 1995, Birtles and Raoult 1996, Renesto et al. 2001). PCR was performed using Syntezza PCR-Ready High Specificity (Syntezza Bioscience, Israel) PCR kit using a programmable conventional thermocycler (Biometra, Goettingen, Germany) as described previously (Kamani et al. 2013).

PCR products were electrophoresed on 1.5% agarose gel stained with ethidium bromide and checked under ultraviolet (UV) light for the size of amplified fragments by comparison to a 100-bp DNA molecular weight marker.

Sequencing

PCR products of appropriate size were purified using Exo-Sap (New England Biolabs Inc., USA) according to the manufacturer's instructions. Sequencing was performed in both directions (forward and reverse) at the Center for Genomic Technologies, Hebrew University of Jerusalem, Israel. DNA sequences obtained were evaluated with MEGA 5.2 software and compared for similarity to sequences deposited in GenBank, using the BLAST program hosted by the National Center for Biotechnology Information (NCBI), National Institutes of Health, Bethesda, MD (www.ncbi.nlm.nih.gov/BLAST). Selected sequences were deposited in GenBank and

TABLE 1. OLIGONUCLEOTIDE PRIMER PAIRS USED IN POLYMERASE CHAIN REACTION AMPLIFICATIONS FOR THE DETECTION OF *BARTONELLA* SPECIES IN BATS AND BAT FLIES

Primer pairs	5'-primer sequence-3'	Target locus	Amplicon (bp)	Reference
BhCS781.p	GGGGACCAGCTCATGGTGG	Citrate synthase gene (<i>gltA</i>)	379	Norman et al. 1995 Birtles and Raoult 1996
BhCS1137.n	AATGCAAAAAGAACAGTAAACA			
CSH1f ^a	GCGAATGAAGCGTGCCTAAA			
BhCS1137.n ^a	AATGCAAAAAGAACAGTAAACA			
600f	CGTGCAACAGAAGATTTAGATC	RNA polymerase beta subunit gene (<i>rpoB</i>)	191	Morick et al. 2009
800r	CGATTTCGCATCATCATTTTC	RNA polymerase beta subunit gene (<i>rpoB</i>) 16S-23S (ITS)	830	Renesto et al. 2001
1400f	CGCATTGGCTTACTTCGTATG			
2300r	GTAGACTGATTAGAACGCTG			
321s	AGATGATGATCCCAAGCCTTCTGG			
493as	TGAACCTCCGACCTCACGCTTATC		208	Maggi et al. 2005

^aPrimer pair used for amplification of *gltA* sequences from DNA extracted from bat blood and flies.

TABLE 2. *BARTONELLA* DNA IN NIGERIAN BATS AND BAT FLIES ACCORDING TO THEIR SPECIES

Bat species/bat fly species	Samples tested	Number positive by real-time PCR		Number positive by conventional PCR	
Bat species		ITS (205bp)	<i>rpoB</i> (190 bp)	<i>gltA</i> (350 bp)	<i>rpoB</i> (830 bp)
<i>Eidolon helvum</i>	79	44	14	9	0
<i>Epomorphorus</i> spp.	30	16	9	3	0
<i>Micropterus</i> spp.	11	6	2	1	0
<i>Chaerephon nigeriae</i>	16	2	0	0	0
<i>Rhinolophus</i> spp.	12	8	3	1	0
Total	148	76	28	14	0
Bat fly species					
<i>Cyclopodia greeffi</i>	24	10	7	7	7

ITS, internal transcribed spacer.

were assigned the following accession numbers KF418806–KF418812 and KF533081–KF533103.

Results

Bats and bat flies

From November, 2012, to March, 2013, 148 bats belonging to five species were collected for this study. *Eidolon helvum* (the straw-colored fruit bat) constituted 53.4% (79/148) of bats captured, followed by *Epomorphorus* spp. (the epauletted fruit bat) (20.3%), *Chaerephon nigeriae* (the free-tailed bat) (10.8%), *Rhinolophus* spp. (the horseshoe bat) (8.1%), and *Micropterus* spp. (the dwarf epauletted fruit bat) (7.4%). All of the bat species captured in this study were found living in mixed colonies except *C. nigeriae*, which was found as a single colony in the ceiling of a school building. A total of 34 (19 male and 15 female) bat flies belonging to the family Nycteribiidae were collected from 24 (16%) of the bats sampled and were all identified as *C. greeffi*, Karsch, 1884. Bat flies were removed from 11 *E. helvum*, three *Micropterus* spp., and five each of *Rhinolophus* spp. and *Epomorphorus* spp.

Bartonella species DNA in bats' blood and bat flies as detected by PCR

When tested by ITS locus and *rpoB* gene-based qPCR, 76/148 (51.4%) and 28/148 (18.9%) bat blood samples were positive, respectively. However, only 14/148 (9.5%) of the same samples were found positive for the *Bartonella* *gltA* gene by conventional PCR. None of the blood samples was positive for the *rpoB* gene by conventional PCR (Table 2).

Bartonella spp. DNA was detected in 10 out of 24 (41.7%) and 7 out of 24 (29.2%) bat flies by real-time PCR targeting the 16S–23S ITS locus and *rpoB* gene, respectively. Similarly, both *gltA* and *rpoB* genes were detected in seven out of 24 (29.2%) of the bat fly samples screened in the study (Table 2).

Several of the bats infested with bat flies at the time of sampling were positive for *Bartonella* spp. DNA targeting 16S–23S ITS locus, *E. helvum* (7/11, 63.6%), *Micropterus* spp. (2/3, 66.7%), *Rhinolophus* spp. (3/5, 60%), and *Epomorphorus* spp. (1/5, 20%). Furthermore, of the infested bats that were positive for *Bartonella* spp. DNA 16S–23S ITS locus, bat flies removed from 71.4% of *E. helvum* and 100% of both *Micropterus* spp. and *Rhinolophus* spp. were also positive. However, the bat flies removed from the only infested and positive *Epomorphorus* spp. was negative for *Bartonella* spp. DNA targeting 16S–23S ITS locus.

Bartonella isolation from bat blood

Bartonella colonies were cultured from 23 of 148 (15.5%) bat blood samples. Prevalence of *Bartonella* spp. culture-positive samples ranged from 0% to 45.5% among the five bat species. *Micropterus* spp. bats had the highest prevalence (45.5%) of *Bartonella* spp. culture positive, followed by *Rhinolophus* spp. (25%), *E. helvum* (15.2%), and *Epomorphorus* spp. (10%). None of the 16 *C. nigeriae* bats was *Bartonella* spp. culture positive. *Micropterus* spp. bats had a significantly higher relative risk of 3.45 for being *Bartonella* spp. culture positive compared to other bat species sampled (Table 3). No significant difference was noticed in the prevalence of *Bartonella* spp. culture-positive bats between

TABLE 3. PREVALENCE OF CULTURE-POSITIVE *BARTONELLA* spp. AMONG BAT SPECIES FROM NIGERIA

Bat species	Number of samples cultured	Number of positive samples	Percent	Relative risk ^a (CI 95%)	p value
<i>Eidolon helvum</i>	79	12	15.2	0.95 (0.45–2.02)	0.90
<i>Epomorphorus</i> spp.	30	3	10.0	0.59 (0.19–1.85)	0.37
<i>Micropterus</i> spp.	11	5	45.5	3.45 (1.59–7.53)	0.0018 ^b
<i>Chaerephon nigeriae</i>	16	0	0	Not defined ^c	1.27
<i>Rhinolophus</i> spp.	12	3	25.0	1.709 (0.58–4.90)	0.33

^aRelative risk was calculated by comparing proportions of positive for each bat species with the number of positive for all other bat species.

^bStatistically significant relative risk.

^cRelative risk is less than 1 but is not defined because of 0 in one of the cells.

CI, confidence interval.

TABLE 4. DISTRIBUTION AND DIVERSITY OF *BARTONELLA* STRAINS FROM BATS AND *BARTONELLA* SPP. FROM BAT FLIES, NIGERIA BASED ON CITRATE SYNTHASE (*GLTA*) GENE FRAGMENTS

Species	Place captured	GenBank first match (no.)	% identity
Bat species			
<i>Eidolon helvum</i>	Bauchi	<i>Bartonella</i> sp. E3-106 (4)	99
<i>Eidolon helvum</i>	Bauchi	<i>Bartonella</i> sp. EW-111 (1)	99
<i>Epomophorus</i> spp.	Bauchi	<i>Bartonella</i> sp. EW-111 (1)	99
<i>Micropterus</i> spp.	Bauchi	<i>Bartonella</i> sp. EW-111 (1)	100
<i>Micropterus</i> spp.	Bauchi	<i>Bartonella</i> sp. EW-111 (1)	99
<i>Micropterus</i> spp.	Bauchi	<i>Bartonella</i> sp. EW-111 (1)	98
<i>Eidolon helvum</i>	Gboko	<i>Bartonella</i> sp. EW-111 (3)	99
<i>Eidolon helvum</i>	Gboko	<i>Bartonella</i> sp. EW-111 (2)	98
<i>Eidolon helvum</i>	Gboko	<i>Bartonella</i> sp. E3-106 (1)	99
<i>Epomophorus</i> spp.	Gboko	<i>Bartonella</i> sp. E3-106 (1)	99
<i>Epomophorus</i> spp.	Gboko	<i>Bartonella</i> sp. E3-106 (1)	98
<i>Micropterus</i> spp.	Gboko	<i>Bartonella</i> sp. E3-106 (1)	99
<i>Micropterus</i> spp.	Gboko	<i>Bartonella</i> sp. E-124 (2)	98
<i>Rhinolophus</i> spp.	Gboko	<i>Bartonella</i> sp. E3-106 (2)	99
<i>Rhinolophus</i> spp.	Gboko	<i>Bartonella</i> sp. EW-111 (1)	99
Bat flies			
<i>Cyclopodia greeffi</i>	Bauchi	<i>Bartonella</i> sp. E3-106 (1)	97
<i>Cyclopodia greeffi</i>	Gboko	<i>Bartonella</i> sp. E3-106 (3)	99
<i>Cyclopodia greeffi</i>	Gboko	<i>Bartonella</i> sp. E-124 (2)	97
<i>Cyclopodia greeffi</i>	Gboko	<i>Bartonella</i> sp. E-124 (1)	99

the two different sampling sites (14.8% vs. 15.9%; $\chi^2=0.03$, $p=0.87$, 95% confidence interval [CI]=0.36–2.33).

Distribution and diversity of *Bartonella* spp. in bats and bat flies based on citrate synthase (*glta*) sequences

Two *glta* genotypes of *Bartonella* spp. with 98%–100% identity to *Bartonella* sp. E3-106 (*Eidolon helvum*, Kenya) and *Bartonella* sp. EW-111 (*Eidolon helvum*, Kenya) were detected and deposited in the GenBank from the three bat genera, *E. helvum*, *Epomophorus* s., and *Micropterus* s. sampled in Bauchi state. In samples collected from four bat genera in Gboko, Benue state, three *Bartonella* spp. *glta* genotypes with 98%–100% identity to *Bartonella* sp. E3-106, *Bartonella* sp. E-124 (*Eidolon helvum*, Kenya), and *Bartonella* sp. EW-111 were detected and deposited in the GenBank (Table 4).

In both study areas, *Bartonella* genotypes with 98%–100% identity with *Bartonella* sp. E3-106 and *Bartonella* sp. EW-111 in the GenBank were detected in the *Bartonella* spp. culture-positive bat blood examined in this study. In addition, a *Bartonella* genotype with 97%–99% identity to *Bartonella* sp. E-124 was also detected in *Micropterus* spp. bats and *C. greeffi* flies captured in Gboko, Benue state, Nigeria (Table 4).

Phylogenetic analysis of *Bartonella* spp. detected in this study shows that they fall into three distinct clusters along with other *Bartonella* genotypes isolated from bats and bat flies from Kenya and Ghana, respectively. The first cluster consists of *Bartonella* spp. genotypes cultured from blood of *E. helvum*, *Rhinolophus* spp. bats, and *C. greeffi* bat flies along with *Bartonella* sp. E3-106 isolated from bats from Kenya and *Bartonella* sp. clone Cg 417-2 from bat flies, Ghana. The second cluster is made up of *Bartonella* genotypes isolated from the blood of *Epomophorus* spp. and *Rhinolophus* spp. bats and *C. greeffi* bat flies along with *Bartonella* sp. E-124 from bats in Kenya. The third cluster

consisted of *Bartonella* spp. cultured from blood of *Epomophorus* spp. and *Micropterus* spp. along with *Bartonella* EW-111 isolated from bats in Kenya and *Bartonella* sp. clone Cg Q98-1 isolated from bat flies, Ghana (Fig. 2).

Discussion

Bats represent about 20% of all mammalian species (Calisher et al. 2006) and are acknowledged to harbor several pathogenic organisms (Gardner et al 1987, Bennett, 2006, van der Poel et al. 2006, Schneider et al. 2009, Dzikwi et al 2010). The present study investigated the presence, prevalence, and genetic diversity of *Bartonella* spp. in five bat species and one bat fly species from Nigeria, West Africa. A total of 132 fruit-eating bats belonging to four genera constituted 89.2% of the study population, while the remaining 16 bats belonged to an insectivorous bat species (*C. nigeriae*). Previous reports documented the presence of bartonellae in bats from Kenya (Kosoy et al. 2010) and bat flies from Ghana, Western Africa (Billeter et al. 2012). To the best of our knowledge, this is the first report of *Bartonella* spp. detection and isolation in bats and bat flies from Nigeria.

The isolation of *Bartonella* spp. in 10%–45.5% of four out of five bat species in this study is an indication of a high prevalence of this bacterium in bat communities in Nigeria. The mean prevalence of 15.5% *Bartonella* spp. culture-positive bats reported in this study is lower than the 33% reported in Guatemala (Bai et al. 2011), 30.2% reported from Kenya, eastern Africa (Kosoy et al. 2010), and 24.1% in Peru (Bai et al. 2012), but closer to 18% from Europe (Gardner et al. 1987). Gardner et al. (1987) initially reported the organisms detected in blood smears in European samples as *Grahamella*, but they are now regarded as *Bartonella* spp., and are higher than the 8.3% reported in United Kingdom bats (Concannon et al. 2005). It is noteworthy that the prevalence found in this study varied greatly (0%–45.5%) between bat

FIG. 2. Maximum likelihood phylogenetic tree of partial (322 bp) *gltA*-*Bartonella* sequences obtained from bats (*) and their nycteribiids (**) from Nigeria, common *Bartonella* species, and *Bartonella* genotypes detected from bats and bat flies from Africa (sequences from GenBank database), with accession numbers in brackets. *Brucella melitensis gltA* sequence was used as an outgroup. Gray boxes indicate clusters that are phylogenetically closely related.

species. Greater differences (0%–88%) were previously reported in Kenyan bat species (Kosoy et al. 2010), Guatemalan bat species (05–90%) (Bai et al. 2011), and Peruvian bat species (0%–100%) (Bai et al. 2012). In our study, *Myotis* spp. had the highest prevalence (45.5%) with a statistically significant increased risk of *Bartonella* spp. infection compared to the other bat species. The prevalence of 15.2% of *Bartonella* spp. in *E. helvum* bats in this study is lower than that of 26% reported in Kenya; however, both studies showed that this species is at lower risk of *Bartonella* spp. infection compared to other bat species. We report a prevalence of 25% and 10% in *Rhinolophus* spp. and *Epomops* spp., respectively, as opposed to 0% reported for both species in bats from Kenya (Kosoy et al. 2010).

The high prevalence of *Bartonella* infection among bats has been attributed to their lifestyle, the colonial structure of

their populations, the close physical contact between individuals, the aggressive interactions, and the typically heavy ectoparasite infestations that probably contribute to the frequent transmission of *Bartonella* spp. among individual animals (Kosoy et al. 2010). The insectivorous bat *C. nigeriae* is a locally abundant species, normally found in ceiling roofs of residential and public buildings such as schools, hospitals, and churches; these bats were all culture negative for *Bartonella* spp., and only two out of 16 bats belonging to this species were found positive by real-time PCR. This finding highlights the need to screen samples by more than one diagnostic method. The low positive prevalence results in *C. nigeriae* can be attributed to the existence of this species in a single colony without cohabitation and intermingling with other bat species as in the case of fruit-eating bats, as was shown in this study.

Bartonella spp. DNA was detected in 29.2% (7/24) *C. greeffi* flies removed from bats in this study. This rate of infection is much lower than that of 66.4% reported for the same bat fly species removed from *E. helvum* bats from Ghana (Billeter et al. 2012), a neighboring country to Nigeria. The lower prevalence found in our study may be due to the low number of flies tested in this study that could be a non-representative sample size or due to differences between the screening tests. *Bartonella* spp. isolated in this study formed three distinct clusters along with *Bartonella* spp. isolated from bats from Africa and deposited in the GenBank (Fig. 2), suggesting apparent congruence between the phylogeny of *Bartonella* and their hosts in Africa (Bai et al. 2012).

Conclusions

In conclusion, a high percentage of bats and bat flies were found positive for *Bartonella* spp. Because the bats sampled in this study were captured in close proximity to human habitations, and due to the fact that bats serve as a protein source or are used for cultural and ritual purposes in some Nigerian communities, there is a need to pay close attention to the role of bats in the epidemiology of *Bartonella* infections in humans and animals in Nigeria. Although, to date, there is no clinical evidence available to suggest whether strains of *Bartonella* spp. found in bats globally are associated with human illness (Kosoy et al. 2010), there is a need for a large-scale surveillance of *Bartonella* spp. in bats across Africa to elucidate the potential role of African bats in public and veterinary health.

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Author Disclosure Statement

No competing financial interests exist.

References

- Bai Y, Kosoy M, Recuenco S, Alvarez D, et al. *Bartonella* spp. in bats, Guatemala. *Emerg Infect Dis* 2011; 17:1269–1272.
- Bai Y, Recuenco S, Gilbert AT, Osikowicz LM, et al. Prevalence and diversity of *Bartonella* spp. in bats in Peru. *Am J Trop Med Hyg* 2012; 87:518–523.
- Bennett M. Bats and human emerging diseases. *Epidemiol Infect* 2006; 134:905–907.
- Bergmans, W. Taxonomy and biogeography of African fruit bats (Mammalia, Megachiroptera). 1. General introduction; material and methods; results: the genus *Epomophorus* Bennett, 1836. *Beaufortia* 1988; 38:75–146.
- Billeter SA, Hayman DTS, Peel AJ, Baker K, et al. *Bartonella* species in bat flies (Diptera: Nycteribiidae) from western Africa. *Parasitology* 2012; 139:324–329.
- Birtles RJ, Raoult D. Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. *Int Journ Syst Bacteriol* 1996; 46:891–897.
- Calisher CH, Childs JE, Field HE, Holmes KV, et al. Bats: Important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 2006; 19:531–545.
- Concannon R, Wynn-Owen K, Simpson VR, Birtles RJ. Molecular characterization of haemoparasites infecting bats (Microchiroptera) in Cornwall, UK. *Parasitology* 2005; 131: 489–496.
- Dzikwi AA, Kuzmin II, Umoh JU, Kwaga JKP, et al. Evidence of Lagos bat virus circulation among Nigerian Fruit Bats. *J Wildlife Dis* 2010; 46:267–271.
- Evans NJ, Bown K, Timofte D, Simpson VR, et al. Fatal borreliosis in bat caused by relapsing fever spirochete, United Kingdom. *Emerg Infect Dis* 2009; 15:1331–1333.
- Gardner RA, Molyneux DH, Stebbings RE. Studies on the prevalence of haematozoa of British bats. *Mammal Rev* 1987; 17:75–80.
- Gibson KE, Rikihisa Y, Zhang C, Martin C. *Neorickettsia risticii* is vertically transmitted in the trematode *Acanthatrium oregonense* and horizontally transmitted to bats. *Environ Microbiol* 2005; 7:203–212.
- Hanson AW. Isolation of spirochaetes from primates and other mammalian species. *Br J Vener Dis* 1970; 46:303–306.
- Kamani J, Morick D, Mumcuoglu KY, Harrus S. Prevalence and diversity of *Bartonella* species in commensal rodents and ectoparasites from Nigeria, West Africa. *PLoS Negl Trop Dis* 2013; 7:e2108.
- Kosoy M, Bai Y, Lynch T, Kuzmin IV, et al. *Bartonella* spp. in bats, Kenya. *Emerg Infect Dis* 2010; 16:1875–1881.
- Kuzmin IV, Bozick B, Guagliardo SA, Kunkel R, et al. Bats, emerging infectious diseases, and the rabies paradigm revisited. *Emerg Health Threats J* 2011; 4:7159.
- Lin JW, Hsu YM, Chomel BB, Lin LK, et al. Identification of novel *Bartonella* spp. in bats and evidence of Asian gray shrew as a new potential reservoir of *Bartonella*. *Vet Microbiol* 2012; 156:119–126.
- Loftis AD, Gill JS, Schrieffer ME, Levin ML, et al. Detection of *Rickettsia*, *Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). *J Med Entomol* 2005; 42:473–480.
- Maggi RG, Breitschwerdt EB. Potential limitations of the 16S-23S rRNA intergenic region for molecular detection of *Bartonella* species. *J Clin Microbiol* 2005; 43:1171–1176.
- Matthias MA, Díaz MM, Campos KJ, Calderon M, et al. Diversity of bat-associated *Leptospira* in the Peruvian Amazon inferred by babesian phylogenetic analysis of 16S ribosomal DNA sequences. *Am J Trop Med Hyg* 2005; 73:964–974.
- Messenger SL, Smith JS, Orciari LA, Yager PA, et al. Emerging pattern of rabies deaths and increased viral infectivity. *Emerg Infect Dis* 2003; 9:151–154.
- Morick D, Baneth G, Avidor B, Kosoy MY, et al. Detection of *Bartonella* spp. in wild rodents in Israel using HRM real-time PCR. *Vet Microbiol* 2009; 139:293–297.
- Norman AF, Regnery R, Jameson P, Greene C, et al. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *J Clin Microbiol* 1995; 33:1797–1803.
- Reeves WK, Loftis AD, Gore JA, Dasch GA. Molecular evidence for novel *Bartonella* species in *Trichobius major* (Diptera: Streblidae) and *Cimex adjunctus* (Hemiptera: Cimicidae) from two southeastern bat caves, U.S.A. *J Vector Ecol* 2005; 30:339–341.

- Reeves WK, Rogers TE, Durden LA, Dasch GA. Association of *Bartonella* with the fleas (Siphonaptera) of rodents and bats using molecular techniques. *J Vector Ecol* 2007; 32:118–122.
- Renesto P, Gouvernet J, Drancourt M, Roux V, et al. Use of rpoB gene analysis for detection and identification of *Bartonella* species. *J Clin Microbiol* 2001; 39:430–437.
- Schipper J, Chanson JS, Chiozza F, Cox NA, et al. The status of the world's land and marine mammals: Diversity, threat and knowledge. *Science* 2008; 322:225–230.
- Schneider MC, Romijn PC, Uieda W, Tamayo H, et al. Rabies transmitted by vampire bats to humans: An emerging zoonotic disease in Latin America? *Rev Panam Salud Publica* 2009; 25:260–269.
- Simmons NB. Order Chiroptera. In: Wilson DE, Reeder DM, eds. *Mammal Species of the World: A Taxonomic and Geographic Reference*, 3rd ed. Baltimore, MD: Johns Hopkins University Press, 2005; 312–529.
- Theodor O. An illustrated catalogue of the Rothschild Collection of Nycteribiidae (Diptera) in the British Museum (Natural History) with keys and short descriptions for the identification of subfamilies, genera, species, and subspecies. London: British Museum (Natural History), 1967; 655 VIII 1 506, p. 898, p. 5, Pl, 6 maps.
- van der Poel WH, Lina PH, Kramps JA. Public health awareness of emerging zoonotic viruses of bats: A European perspective. *Vector Borne Zoonotic Dis* 2006; 6:315–324.
- Wang LF, Walker PJ, Poon LLM. Mass extinctions, biodiversity and mitochondrial function: Are bats 'special' as reservoirs for emerging viruses? *Curr Opin Virol* 2011; 1:649–657.
- Wong S, Lau S, Woo P, Yuen KY. Bats as a continuing source of emerging infections in humans. *Rev Med Virol* 2007; 17:67–91.

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