Diversity of Bartonella spp. in Bats, Southern Vietnam

Pham Hong Anh, Nguyen Van Cuong, Nguyen Truong Son, Ngo Tri Tue, Michael Kosoy, Mark E.J. Woolhouse, Stephen Baker, Juliet E. Bryant, Guy Thwaites, Juan J. Carrique-Mas, Maia A. Rabaa

Author affiliations: Oxford University, Ho Chi Minh City, Vietnam (P.H. Anh, N. Van Cuong, N.T. Son, N.T. Tue, S. Baker, J.E. Bryant, G. Thwaites, J.J. Carrique-Mas, M.A. Rabaa); Vietnam Academy of Sciences and Technology, Hanoi, Vietnam (N.T. Son); Centers for Disease Control and Prevention Fort Collins, Colorado, USA (M. Kosoy); University of Edinburgh, Edinburgh, Scotland, UK (M.E.J. Woolhouse, M.A. Rabaa); London School of Hygiene and Tropical Medicine, London, UK (S. Baker); Oxford University, Oxford, UK (S. Baker, J.E. Bryant, G. Thwaites, J.J. Carrique-Mas)

DOI: http://dx.doi.org/10.3201/eid2107.141760

To the Editor: To investigate bats as potential reservoirs for Bartonella spp. in Vietnam, we screened a range of bat species to determine the prevalence and genetic diversity of Bartonella spp. in bat populations in southern Vietnam. In a study of bat biodiversity in southern Vietnam, 60 bats were trapped at 6 sites in Dong Nai Nature Reserve and Cat Tien National Park, Vietnam, in May 2013. Bats were trapped by using mist nets and harp traps set at ground level, and were euthanized by using isoflurane for cataloguing at the Vietnam Academy of Sciences and Technology, Hanoi. Blood specimens were collected by cardiac puncture, and external measurements were recorded. Bats were snap-frozen until morphological (1,2); trapped bats represented 10 species belonging to 5 genera. All species have been given a conservation status of least concern (http://www.iucnredlist.org/).

Total nucleic acid was extracted from blood samples by using the MagNAPure automated nucleic acid extraction system (Roche, Basel, Switzerland). Extracted nucleic acid was subjected to conventional PCR to detect Bartonella spp. DNA by using primers specific for the citrate synthase A gene (3). A positive PCR result was determined by amplification of a 729-bp fragment. Twenty-one (35.0%) of 60 blood specimens had a result consistent with presence of Bartonella spp. (Table). Among insectivorous or carnivorous bats, Bartonella prevalence was 20 (45.5%) of 44 compared with 1 (6.2%) of 16 fruit-eating bats (χ² = 6.3, p = 0.01). The prevalence of Bartonella spp. did not differ between sampling locations (Table) or by estimated age of the bat (determined by deviation above or below the median tibial length of each species); prevalence was 33.3% (9/27) in younger bats and 36.4% (12/33) in older bats.

DNA sequences from the 21 PCR citrate synthase A gene amplicons (GenBank accession nos. KP100340–KP100360) were subjected to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to assess sequence similarity. Potentially novel Bartonella phylogroups were identified as having <96% sequence similarity with all publicly available sequences in GenBank (4). The sequences were then manually aligned with those of a representative sample of Bartonella spp. and trimmed to the 327-nt region (positions 801–1127) commonly used for taxonomic classification (4). A neighbor-joining tree was constructed by using the Hasegawa–Kishino–Yano plus gamma model of nucleotide substitution in Geneious version 7.1.7 with 1,000 bootstrap replications (5).

Sequence analysis identified 10 distinct Bartonella phylogroups (I–X) among 21 Bartonella-positive blood samples from bats in Vietnam (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/7/14-1760-Techapp1.pdf). Nine of these phylogroups showed <96% sequence similarity to all previously identified Bartonella sequences, suggesting they might belong to new Bartonella species. Bartonella spp. in Rhinolophus spp. bats were classified into phylogroups I, III, VIII, IX, and X. Phylogroups II and VII were detected in samples from Hipposideros spp. bats, and phylogroups IV and V were detected in Megaderma spp. bats. Phylogroup VI was detected only in a Megaerops spp. bat.

Although 9 lineages (I, III–X) were novel, phylogroup II was identified in 4 Hipposideros spp. bats and showed 96.3%–97.2% similarity to Bartonella spp. isolated from a bat fly found on a Hipposideros spp. host in Malaysia (GenBank accession no. JX416238). This similarity might suggest widespread distribution of this Bartonella spp. lineage in Hipposideros spp. bats or their ectoparasites in Southeast Asia. Additional genetic characterization of strains is needed to determine whether any of these novel phylogroups represent new species and to investigate their evolutionary and ecological relationships with other Bartonella spp. identified in Vietnam and elsewhere.

The primary observation in this study was detection of Bartonella spp. (by DNA amplification) in bats in southern Vietnam at a prevalence of 35.0%, which is comparable with that reported in Kenya (30.2%) and Guatemala (33.0%) (Table) (6,7). However, the use of conventional PCR in this study might underestimate the true prevalence. Although high prevalences have been proposed to be caused by persistent infection of bats with Bartonella spp., our findings indicate no increase in prevalence by age of bat, which would be expected if persistent infection were common. This finding, and detection of multiple lineages infecting individual bat species, may instead reflect high levels of transmission within and between bat species.
caused by crowded roosting areas and sharing of roosts by multiple species. This behavior provides opportunities for transmission of Bartonella bacteria or exchange of infected ectoparasites, such as Cyclopodia spp. (8), although the precise roles of these 2 processes are unknown.

Although no human cases of Bartonella spp. infection have been reported in Vietnam, Bartonella spp. have been identified in bearded humans elsewhere in Southeast Asia (9) and are also common in rats in southern Vietnam (10). Because close contact with bats (i.e., through manure farming and consumption of bat meat) and potential arthropod vectors (i.e., through handling and consumption of fruit) is common in parts of Vietnam, targeted screening of bats and their human contacts might improve our understanding of the zoonotic potential of these bacteria and their potential effect on public health.

This study was supported by a strategic award from the Wellcome Trust of Great Britain (WT/093724).

Ms. Pham is a research assistant at the Oxford University Clinical Research Unit in Ho Chi Minh City, Vietnam. Her primary research interests focus on characterizing the diversity and spread of potential agents of zoonotic disease in domestic and wild animal populations across Vietnam.

References

1. Kruskop SV. Bats of Vietnam. Checklist and an identification manual. Moscow: KMK Scientific Press; 2013.
2. Francis CM. A guide to the mammals of Southeast Asia. Princeton (NJ): Princeton University Press; 2008.
3. Billeter SA, Hayman DT, Peel AJ, Baker K, Wood JL, Cunningham A, et al. Bartonella species in bat flies (Diptera: Nycteribiidae) from western Africa. Parasitology. 2012;139:324–9. http://dx.doi.org/10.1017/S0031182011002113
4. La Scola B, Zeaiter Z, Khamis A, Raoult D. Gene-sequence-based criteria for species definition in bacteriology: the Bartonella paradigm. Trends Microbiol. 2003;11:318–21. http://dx.doi.org/10.1016/S0966-842X(03)00143-4
5. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28:1647–9. http://dx.doi.org/10.1093/bioinformatics/bts199
6. Kosoy M, Bai Y, Lynch T, Kazmin IV, Niezgoda M, Franka R, et al. Bartonella spp. in bats, Kenya. Emerg Infect Dis. 2010;16:1875–81. http://dx.doi.org/10.3201/eid1610.100601
7. Bai Y, Kosoy M, Recuenco S, Alvarez D, Moran D, Turnelle A, et al. Bartonella spp. in bats, Guatemala. Emerg Infect Dis. 2011;17:1269–72. http://dx.doi.org/10.3201/eid1707.101867
8. Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranaivoson HC, Zhu Q, et al. Bartonella spp. in fruit bats and blood-feeding ectoparasites in Madagascar. PLoS Negl Trop Dis. 2015; 9:e0003532. http://dx.doi.org/10.1371/journal.pntd.0003532
9. Kosoy M, Morway C, Sheff KW, Bai Y, Colborn J, Chalcraft L, et al. Bartonella tamae sp. nov., a newly recognized pathogen isolated from three human patients from Thailand. J Clin Microbiol. 2008;46:772–5. http://dx.doi.org/10.1128/JCM.02120-07
10. Loan HK, Van Cuong N, Takhampunya R, Klangthong K, Osikowicz L, Kiet BT, et al. Bartonella spp. and trombiculid mites of bats from the Mekong Delta of Vietnam. Vector Borne Zoonotic Dis. 2015;15:40–7. http://dx.doi.org/10.1089/vbz.2014.1604

Address for correspondence: Juan J. Carrique-Mas, Clinical Research Unit, Oxford University 764 Vo Van Kiet, Ward 1, District 5, TP, Ho Chi Minh City, Vietnam; email: jcarrique-mas@oucru.org

Seropositivity for Avian Influenza H6 Virus among Humans, China

Li Xin, Tian Bai, Jian Fang Zhou, Yong Kun Chen, Xiao Dan Li, Wen Fei Zhu, Yan Li, Jing Tang, Tao Chen, Kun Qin, Jing Hong Shi, Rong Bao Gao, Da Yan Wang, Ji Ming Chen, Yue Long Shu

Author affiliations: National Institute of Viral Disease Control and Prevention, Beijing, China (L. Xin, T. Bai, J.F. Zhou, Y.K. Chen, X.D. Li, W.F. Zhu, Y. Li, J. Tang, T. Chen, K. Qin, J.H. Shi, R.B. Gao, D.Y. Wang, Y.L. Shu); China Animal Health and Epidemiology Center, Qingdao, China (J.M. Chen)

DOI: http://dx.doi.org/10.3201/eid2107.150135

Table. Prevalence of Bartonella spp. in bats from 2 sites in Dong Nai, Vietnam, 2013

| Bat species                  | Cat Tien National Park | Dong Nai Nature Reserve | Total   |
|------------------------------|------------------------|-------------------------|---------|
| Cynopterus sphinx*           | 0/0                    | 0/14                    | 0/14 (0) |
| Hipposideros armiger†         | 2/6                    | 0/0                     | 2/6 (33.3) |
| Hipposideros larvatus†        | 3/5                    | 0/0                     | 3/5 (60) |
| Macroaglaeus niphanae*        | 0/0                    | ½                       | ½ (50)  |
| Megaderma spasma†             | 0/0                    | 1/2                     | 1/2 (50) |
| Megaderma lyra†               | 1/1                    | 0/0                     | 1/1 (100) |
| Rhinolophus acuminatus†       | 0/0                    | 9/17                    | 9/17 (52.9) |
| Rhinolophus chaeli†           | 2/5                    | 0/0                     | 2/5 (40) |
| Rhinolophus sinicus†          | 0/3                    | 2/4                     | 2/7 (28.6) |
| Rhinolophus lucus†            | 0/1                    | 0/0                     | 0/1 (0)  |
| Total                        | 8/21 (38.1)            | 13/39 (33.3)            | 21/60 (35) |

*Fruit-eating.  †Insectivorous.  ‡Carnivorous.