Bartonella spp. and Rickettsia felis in Fleas, Democratic Republic of Congo

To the Editor: Bartonella and Rickettsia species are pathogens of humans and domestic mammals that may be transmitted by fleas and other arthropods. Rickettsia felis causes fleaborne spotted fever in humans who come into contact with flea-infested domestic and peridomestic animals; worldwide distribution of this pathogen in ectoparasites and mammals makes it an emerging threat to human health (1,2). Likewise, species of the genus Bartonella are associated with an increasing array of human diseases, including trench fever, cat-scratch disease, and endocarditis in immunocompetent patients, and bacillary angiomatosis and peliosis hepatitis in immunocompromised patients (3–5). Although Bartonella spp. and R. felis appear to be globally distributed, their presence in the Democratic Republic of Congo (DRC) has not been previously documented.

Off-host Pulex irritans, Tunga penetrans, Ctenocephalides felis strongylus, Echidnophaga gallinacea, and Xenopsylla brasiliensis were collected in the Ituri district of northeastern DRC from March through April 2007, during an investigation of a plague outbreak. Our investigation area was limited to 4 villages: Djalusene and Kpandruma, which had confirmed plague patients, and Wanyale and Zaa, which had several suspect cases (6). We collected fleas by using a kerosene lamp hung above a 45-cm diameter tray containing water (7). Captured fleas were identified using a dissecting microscope and standard morphologic keys, sorted into vials by species and locality, and preserved in 70% ethanol (7). Fleas were separated into 193 pools (2–5 fleas per pool), triturated for 10 minutes; the resultant flea triturate was centrifuged at 3,000 rpm for 10 minutes to collect flea tissue. DNA was then obtained by using the DNaseq Blood and Tissue Kit (QIAGEN, Valencia, CA, USA).

Bartonella DNA was detected by PCR amplifying a 379-bp fragment of the citrate synthase gene (gltA) (8). For Rickettsia typhi and R. felis, a real-time multiplex PCR assay targeting a conserved fragment of gltA was used (unpub. data). All assays were run in duplicate, and positive and negative controls were included in all assays. Amplicons were purified with the QIAquick PCR purification kit (QIAGEN) and sequenced in both directions by using a BigDye sequencing kit (Applied Biosystems, Foster City, CA, USA) with the same primers used for PCR amplification. Resultant sequences of Bartonella spp. were aligned with MegAlign using the Clustal algorithm (DNASTAR, Inc., Madison, WI, USA), and compared with reference sequences obtained from GenBank.

Although Yersinia DNA and R. typhi were not detected, 89 of the 193 pools were PCR positive for either Bartonella spp. or R. felis (Table). Using the Microsoft Excel Add-In PooledInRate software (Redmond, WA, USA; www.cdc.gov/ncidod/dvbid/westnile/software.htm), we calculated an estimated infection rate of 10.72% (95% confidence interval [CI] 8.52–13.31) for R. felis, 3.66% for Bartonella species, and 0.91% (95% CI 0.40–1.78) for both Bartonella spp. and R. felis (Table).

Phylogenetic analysis indicated several Bartonella spp. in fleas that were closely aligned with pathogenic Bartonella spp., including B. vinsonii, Candidatus B. rochalimaea, and B. clarridgeiae (data not shown). Moreover, Bartonella from 3 pools of P. irritans demonstrated only 1.8% to 2.4% divergence to B. vinsonii sub-species arupensis isolated from a human patient in Wyoming, USA. Likewise, sequences of Bartonella from
This report suggests that *Bartonella* spp. and *R. felis* exists in fleas within the DRC. In addition, we report *Bartonella* spp. and *R. felis* DNA in *T. penetrans* fleas and *R. felis* DNA in *E. gallinacea* fleas, vectors not previously associated with these pathogens. Co-infections were also observed in *T. penetrans*, *P. irritans*, and *C. felis* fleas, suggesting a common vector or mammalian host shared by *R. felis* and *Bartonella* spp. Flea feedings occur intermittently and on potentially different hosts, thus the vectors described here may acquire multiple bacterial strains for transmission to humans (8). Moreover, PCR assays targeting the cytochrome B gene indicated human blood in the flea pools, demonstrating dual infection (data not shown); this finding shows that the flea species recovered are capable of feeding on humans, have a broad host range, and are capable of transmitting disease to humans (9).

*Bartonella* spp. and *R. felis* have been detected previously in fleas within northern and sub-Saharan Africa (10). The presence of *Bartonella* spp. and *R. felis* in the fleas is important because they were collected in close contact with humans at risk for multiple exposures within households. Our results suggest that both *R. felis* and *Bartonella* spp. are prevalent in this region of the DRC and should be included in the differential diagnosis of potential flea-borne infections in this region of sub-Saharan Africa.

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**Table. Detection of *Bartonella* spp. and *Rickettsia* spp. DNA in fleas collected in the Democratic Republic of Congo**

| Village     | Flea species† | Sample no. | Bartonella spp.‡ | Rickettsia spp.‡ |
|-------------|---------------|------------|------------------|-----------------|
| Kpandruma   | *Pulex irritans* | 1–2        | *B. clarridgeiae* | *R. felis*      |
|             | *P. irritans*  | 3–5        | *B. vinsonii*     |                 |
|             | *P. irritans*  | 6          | *B. vinsonii*     | *R. felis*      |
|             | *P. irritans*  | 7–8        | Unique            |                 |
|             | *P. irritans*  | 9–24       | *R. felis*        |                 |
|             | *Xenopsylla brasiliensis* | 25 | *R. felis* |                 |
|             | *Tunga penetrans* | 26 | *R. felis* |                 |
| Djalusene   | *P. irritans*  | 27         | *B. vinsonii*     |                 |
|             | *T. penetrans* | 28         | Identical to EU549693 |                 |
|             | *P. irritans*  | 29         | *B. clarridgeiae* |                 |
|             | *P. irritans*  | 30–34      | *Candidatus B. rochalmae* |         |
| Ctenocephalides felisstrongylos | 35 | *B. clarridgeiae* | *R. felis* | |
|             | *T. penetrans* | 36         | *Candidatus B. rochalmae* | *R. felis* |
|             | *P. irritans*  | 37         | *Candidatus B. rochalmae* | *R. felis* |
|             | *T. penetrans* | 38–39      | *R. felis*        |                 |
|             | *P. irritans*  | 40–45      | *R. felis*        |                 |
|             | *C. felis strongylus/P. irritans* | 46 | *R. felis* | |
|             | *T. penetrans/Echidnophaga gallinacea* | 47 | *R. felis* | |
|             | *E. gallinacea* | 48 | *R. felis* | |
| Wanyele     | *P. irritans*  | 49         | *B. vinsonii*     | *R. felis*      |
|             | *C. felis strongylus* | 50 | *Candidatus B. rochalmae* | *R. felis* |
|             | *E. gallinacea* | 51 | *R. felis* | |
|             | *C. felis strongylus* | 52–57 | *R. felis* | |
|             | *T. penetrans* | 58–59      | *R. felis*        |                 |
|             | *P. irritans*  | 60–63      | *R. felis*        |                 |
| Zaa         | *P. irritans*  | 64         | *B. vinsonii*     |                 |
|             | *P. irritans*  | 65–66      | *Candidatus B. rochalmae* | *R. felis* |
|             | *P. irritans*  | 67         | *Candidatus B. rochalmae* | *R. felis* |
|             | *C. felis strongylus* | 68 | *B. clarridgeiae* | |
|             | *P. irritans*  | 69–87      | *R. felis*        |                 |
|             | *E. gallinacea* | 88 | *R. felis* | |
|             | *C. felis strongylus/P. irritans* | 89 | *R. felis* | |

†Boldface indicates dual infection. An expanded version of this table showing all species detected is available online (www.cdc.gov/EID/content/14/12/1972-T.htm).

‡Identified by using standard taxonomic keys.

†Detected by PCR as described in Methods.
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Antibodies to Nipah or Nipah-like Viruses in Bats, China

To the Editor: Hendra virus (HeV) and Nipah virus (NiV), the only known members of the genus Henipavirus, are 2 emerging paramyxoviruses that are highly pathogenic in a variety of vertebrate animals, including humans (1). Since the initial discovery of the viruses in Australia and Malaysia (2,3), sporadic HeV outbreaks have been reported from 1995 to 2007 in Australia (4), and regular NiV outbreaks have occurred in Bangladesh (5) and India (6). Numerous frugivorous bat species (genus Pteropus), and some insectivorous bat species have been found to be reservoir hosts of henipaviruses in Australia and Asian countries (7–9).

In this study conducted during 2004–2007, bats were trapped within their natural habitat from 10 provinces in mainland People’s Republic of China. Serum, pharyngeal, and fecal swab samples were collected and stored as described previously (10). An ELISA was developed to detect antibodies to the NiV nucleocapsid (N) and attachment glycoprotein (G) proteins. For confirmation, ELISA-positive samples were tested by using Western blot against a recombinant NiV G fragment (aa 71–193) fused with the maltose-binding protein. Virus neutralization tests were conducted with live NiV and HeV under Biosafety Level 4 containment in Australia. In addition, a surrogate neutralization test was developed by using recombinant env–HIV-1, pseudotyped with NiV G and F. RNA was extracted by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Reverse transcription–PCR (RT-PCR) was performed by using primers against the NiV N gene as described previously (3).

In total, 692 bat serum specimens were screened for antibody to NiV N or G protein (or both) by ELISA, and 33 were positive (online Appendix Table, available from www.cdc.gov/EID/content/14/12/1974-appT.htm). These specimens were from 9 of the 23 bat species examined in this study. Of the 33 serum samples reactive in ELISA, 25 with sufficient quantity left were further tested by Western blot, and 17 of 25 serum samples were reactive with MBP-NiV G fusion fragment, but not with the control MBP. None of the samples inhibited entry of NiV F/G-pseudotyped virus or neutralized either HeV or NiV. No NiV-specific RNA was detected by RT-PCR among 479 fecal swab samples and 67 throat swab samples tested; therefore, virus isolation was not attempted.

This study systematically investigated NiV presence among bats in China. The detection of henipavirus antibody suggests that several bat species have been exposed to NiV or a closely related virus. The prevalence of antibody was especially prominent among Myotis species from Yunnan.