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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 flea-borne 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 DNeasy 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 *glt*A 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 by 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 PooledInfRate 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* subspecies *arupensis* isolated from a human patient in Wyoming, USA. Likewise, sequences of *Bartonella* from

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*	
	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. rochalimae*	
	Ctenocephalides felis strongylus	**35**	***B. clarridgeiae***	***R. felis***
	T. penetrans	**36**	***Candidatus B. rochalimae***	***R. felis***
	P. irritans	**37**	***Candidatus B. rochalimae***	***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. rochalimae***	***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. rochalimae*	
	P. irritans	**67**	***Candidatus B. rochalimae***	***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.

1 pool of *T. penetrans* and 1 pool of *P. irritans* were 100% identical to *Bartonella* isolated from a *Neotoma mexicana* wood rat (GenBank accession no. AF110312); a sequence obtained from 1 flea pool of *T. penetrans* was 100% identical to the *glt*A *Bartonella* sequence found in *Orchopeas sexdentatus,* collected from *Neotoma micropus* in New Mexico, USA (data not shown). This finding indicates a new *Bartonella* species with multiple rodent origins and a more ubiquitous global dissemination than previously determined. Our results also demonstrate the previously unreported detection of *R. felis* in *P. irritans*, *E. gallinacea*, *X. brasiliensis*, and *T. penetrans* flea species.

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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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 insectivous 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 Biosaftey 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 QIA amp 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