

were conducted by using MEGA version 4.1 (www.megasoftware.net). The partial *lppA* gene nucleotide sequences of isolates GN407 and GN408 were submitted to GenBank under accession nos. HQ699892 and HQ699893, respectively.

PCR amplifications of the 2 joint fluids and their cultures were positive for *M. leachii*. When we compared the complete 16S rRNA gene and the partial *lppA* gene, the 2 isolates from the same epizootic shared 100% nt identity. For 16S rRNA gene, the isolates shared 99.9%, 99.9%, and 99.7% nt identities to *M. leachii* PG50, *M. capricolum* subsp. *capricolum*, and *M. mycoides* subsp. *mycoides* small colony, respectively. For partial *lppA* gene, the isolates shared 99.6%, 95.1%, and 69.6% nt identities to *M. leachii* PG50, *M. mycoides* subsp. *mycoides* small colony, and *M. capricolum* subsp. *capricolum*, respectively.

Intraarticular inoculation of the passage cultures successfully reproduced the polyarthritis in calves 1 month of age. Thus, there are notable similarities between our findings and those reported in Australia (3). Multidisciplinary procedures, including clinical assessment and comprehensive laboratory investigations of affected calves, were used to identify the etiologic agent. The results showed that the outbreak of the serious polyarthritis in calves was caused by *M. leachii*.

Our detection of *M. leachii* in China confirms a wider geographic presence of this type of *Mycoplasma* spp. in cattle and suggests *M. leachii* is common and potentially distributed worldwide. Currently, the source of *M. leachii* infection and its means of spread have not been established. However, our epidemiologic and clinical investigations indicated clear evidence of seminal infection because all calves with arthritis were from dams that were fertilized by using the same batch of semen, and cows in the

same herd that were fertilized by using a different batch of semen delivered healthy calves. More epidemiologic, molecular, and pathogenic studies are required to determine the relevance, distribution, importance, and diversity of *M. leachii* in cattle.

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## *Bartonella clarridgeiae* in Fleas, Tahiti, French Polynesia

**To the Editor:** *Bartonella* species are small, gram-negative, fastidious, and hemotropic emerging pathogens that cause various human diseases and circulate between a large variety of mammalian and arthropod vectors. More than 30 *Bartonella* species have been isolated from humans as well as from wild and domestic animals worldwide (1). *B. clarridgeiae* was suggested to be a minor causative agent of cat-scratch disease (CSD) in humans, however, this suggestion remains controversial. Usually, the agent of CSD is *B. henselae* and its principal reservoir is domestic cats (*Felis catus*) (1,2). The principal vector of these 2 species is the cat flea (*Ctenocephalides felis*) (3,4).

We report *Bartonella* species in fleas collected from cats and dogs in Tahiti, French Polynesia.

In October 2009, fleas were collected from 1 cat and 9 dogs in Papeete, capital of Tahiti Island, French Polynesia. Fleas collected were kept in 70% ethanol and sent to the military veterinary service in Marseille, France; these fleas were later sent to Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes in Marseilles. The fleas were identified phenotypically by using current taxonomic criteria. DNA from fleas and negative control DNA from noninfected laboratory lice were extracted by using a QIAamp Tissue Kit (QIAGEN, Hilden, Germany), as described (3).

Flea samples were tested for *Bartonella* spp. DNA by using the 7900 HT Fast Quantitative Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and primers and Taqman probes specific for the 16S–23S rRNA gene intergenic spacer region as described (5). Fleas were considered positive when cycle threshold was  $\leq 30$ . All positive fleas at screening were confirmed by using standard *Bartonella* PCR and sequencing of partial internal transcribed spacer gene fragments by using primers URBarto1 and URBarto2, as described (3). *B. elizabethae* DNA was used as positive control. DNA sequencing reagents were obtained with BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI PRISM; Applied Biosystems). The sequences were assembled in Sequencher 4.2 (GeneCodes 2003; www.genecodes.com) and were compared with *Bartonella* sequences available in GenBank.

Overall, 81 fleas were collected from 1 cat (13 fleas) and 9 dogs (68 fleas). All 81 fleas collected were morphologically identified as *C. felis*.

Sample fleas were collected from animals visiting a veterinary clinic for neutering or vaccinations. The overall

rate of *Bartonella*-positive fleas by molecular screening with real-time PCR was 7.4% (6/81): 6 fleas from the cat (6/13) and none from a dog (0/68). These positive samples were confirmed after intergenic spacer PCR amplification and sequencing with sequences at 100% identity with *B. clarridgeiae* (GenBank accession no. EU589237).

*B. clarridgeiae* was first isolated from the pet cat of an HIV-positive patient in the United States (6). However, *B. clarridgeiae* has never been isolated or detected by molecular methods in humans, and thus its implication as a human pathogen remains controversial. The presence of *B. clarridgeiae* antibodies has been reported in a suspected case of CSD and in a patient with a chest-wall abscess (4). However, *B. clarridgeiae* has been detected on fleas from various continents, including Europe, Asia, North America (1), Africa; New Zealand, and recently from New Caledonia (7).

In France, several studies have reported the molecular detection of *B. clarridgeiae* in the blood of a cat or in cat fleas (*C. felis*), indicating the potential role of fleas as vectors of this organism (8,9). Prevalence of this bacterium in cat fleas may vary and be as high as 67.9% in cat fleas from France (3). Moreover, DNA of *B. henselae* and *B. clarridgeiae* has been reported from cat fleas from New Zealand (10). Similarly, co-infection with *B. clarridgeiae* and *B. henselae* has been reported in domestic cats from Europe and Asia (1). In our study, all *Bartonella* spp.–positive fleas harbored *B. clarridgeiae* only; all were obtained from cats and none from dogs, similar to findings reported from New Zealand (10), although *B. clarridgeiae* has been reported from a flea on a dog in Taiwan (2).

Papeete, the capital of Tahiti, is located in the South Pacific Ocean, and remains one of the most visited areas by tourists from all over the world. There

are many stray cats and dogs in Tahiti that may be infected with *Bartonella* species and thus serve as a reservoir for these pathogens. Our result confirms the presence of *B. clarridgeiae* in Tahiti and is a warning of the presence of flea-borne bartonellosis and the potential risk of *B. clarridgeiae* or other flea-borne diseases for humans exposed to cat fleas.

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## Bocavirus in Children with Respiratory Tract Infections

**To the Editor:** Four species of human bocavirus (HBoV1–4) have been identified since 2005 (1–4). Several reports have documented that HBoV1 are prevalent in respiratory tract samples. Although there may be many asymptomatic carriers, HBoV1 has been shown to cause respiratory tract diseases (1,5,6). HBoV2 has mainly been detected in fecal samples and has been linked to gastroenteritis (3,7,8). HBoV3 and HBoV4 have

recently also been detected in fecal samples (3,4), although no link to disease has been established for these 2 species.

In this study, we identified and characterized 3 HBoV species (HBoV1–3) detected in respiratory samples. Nasopharyngeal aspirates were collected from 1,238 children (784 boys and 454 girls) with acute lower respiratory tract infections hospitalized in Beijing Children's Hospital from March 2008 through July 2010. Patients' ages ranged from 1 month to 9 years (median 10.0 months, mean 32.1 months).

Viral nucleic acid was extracted from nasopharyngeal aspirates by using the NucliSens easyMAG system (bioMérieux, Marcy l'Etoile, France). We screened for HBoV1–4 by nested PCR with touch-down procedure using primers targeting the viral proteins (VP) 1/2 region (4). For HBoV-positive samples, we then quantified viral loads by real-time PCR (online Technical Appendix, [www.cdc.gov/EID/content/17/9/110078-Techapp.pdf](http://www.cdc.gov/EID/content/17/9/110078-Techapp.pdf)). Additional viral infections were identified in all screened specimens as described (9). To avoid contamination, the PCR process (including master mixture preparation, nucleic acid extraction, reaction installation, and DNA amplifications) were performed in separate dedicated areas. Strict controls were used during the process of nucleic acid extraction and PCR analyses to monitor contamination. All PCR products were verified by sequence analysis.

We found 141 positive samples for HBoVs (11.4%) from patients ranging in age from 1 to 132 months (median 12.0 months, mean 19.8 months) (GenBank accession nos. HQ871520–HQ871650 and HQ871664–HQ871673). Among these samples, 131 (10.6%; patient ages 1–132 months, median 12 months, mean 19 months) were positive for HBoV1, 5 (0.4%; patients 1–113

months of age, median 7.2 months, mean 29.2 months) for HBoV2, and 5 (0.4%; 1–108 months of age, median 12.0 months, mean 30.4 months) for HBoV3 on the basis of sequence alignment and phylogenetic analysis of PCR amplicons. No specimens were positive for HBoV4. The number of samples positive for HBoV1, -2, and -3 in children  $\leq 5$  years old was 124/131 (94.7%), 4/5 (80%), and 4/5 (80%), respectively. Additional respiratory viruses were co-detected in 120/141 (85.1%) HBoV-positive patients (Table). An unanticipated finding was that real-time PCR only detected 1/5 positive sample for HBoV2 (viral load  $4.87 \times 10^9$  copies/mL) and 2/5 positive samples for HBoV3 (viral load  $2.59 \times 10^4$  and  $4.1 \times 10^2$  copies/mL). In contrast, we detected viral loads of  $8.35 \times 10^4$  to  $1.28 \times 10^9$  copies/mL in 5 randomly selected HBoV-1-positive samples.

The clinical diagnoses of patients providing HBoV-positive samples included pneumonia (63.1%), bronchitis (14.9%), bronchopneumonia (12.8%), and acute asthmatic bronchopneumonia (9.2%). No clinically significant differences were found between the signs and symptoms of patients with HBoV1, -2, and -3 (cough, sputum production, fever, runny nose, wheezing, and diarrhea). For patients positive for HBoV3, the major diagnoses were pneumonia (2/5), bronchopneumonia (2/5), and acute asthmatic bronchopneumonia (1/5). Main signs and symptoms included cough (5/5), wheezing (4/5), sputum production (3/5), fever (3/5), runny nose (1/5), and diarrhea (1/5). For patients positive for HBoV2, the diagnoses were pneumonia (4/5) and bronchopneumonia (1/5), and main signs and symptoms included cough (5/5), sputum production (4/5), wheezing (3/5), fever (3/5), and runny nose (2/5) (Table).

HBoV2 and HBoV3 were detected sporadically during the study. HBoV3 was detected in samples collected in