

Hillerød, Denmark) by agglutination with latex beads and type-specific serum and by microscopic determination of capsule swelling with type-specific serum (SSI Diagnostika, Hillerød, Denmark), according to the manufacturer's instructions. These methods gave concordant results. Etests (bioMérieux, Solna, Sweden) demonstrated that the isolate was sensitive to all antimicrobial drugs tested; MIC was 0.125 mg/L for cefotaxim and 0.016 mg/L for benzylpenicillin. The isolate was tested for known virulence-associated genes *sly*, *mrp*, and *epf* with PCR, as described (9). PCR fragments of predicted sizes were obtained with primer hybridizing to *sly* and *mrp* but not with primers hybridizing to *epf*. A serotype 2 isolate (kindly provided by Susanne Sauer at SSI) was used as a positive control for the *epf* primers.

S. suis is an emerging human pathogen, but reports of human infections caused by serotypes other than serotype 2 remain scarce. This case demonstrates that *S. suis* of serotype 5, which is a serotype routinely isolated from deceased pigs (10), can cause invasive infections in humans. The course of the described infection was relatively favorable, and the patient did not show signs of a systemic inflammatory response syndrome or of meningitis. Preexisting osteoarthritis of the right hip might have had diminished local defenses, thereby enabling colonization of the hip area by bacteria that had entered the bloodstream through the wound on the patient's hand. The isolate we recovered possessed *sly* and *mrp* genes, which encode the virulence-associated suilysin and muraminidase-released proteins, but clearly other factors are also of importance for determining the virulence of individual *S. suis* isolates.

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***Bartonella henselae* and *B. koehlerae* DNA in Birds**

To the Editor: Bartonellosis, a globally emerging vector-borne zoonotic bacterial disease, is caused by hemotropic, gram-negative, aerobic, facultative intracellular *Bartonella* spp. (1). Of the 30 *Bartonella* species/subspecies, 17 have been associated with human infections (2,3). Each species has a reservoir host(s), within which the bacteria can cause intraerythrocytic bacteremia with few or no clinical signs of illness (1,3); the bacteria are transmitted by hematophagous arthropod vectors (1). Various *Bartonella* spp. have been identified in domestic and wild animals, including canids, deer, cattle, rodents, and marine mammals (1,4). *Bartonella* DNA from the blood of loggerhead sea turtles (*Caretta caretta*) has been PCR amplified and sequenced (5); the fact that *Bartonella* DNA was found suggests the possibility that persistent blood-borne infection can occur in nonmammals and that the host range for *Bartonella* spp. may be larger than anticipated.

Growing evidence suggests that wild birds play key roles in the maintenance and movement of zoonotic pathogens such as tick-borne encephalitis virus and *Borrelia* and *Rickettsia* spp. (6–9). *Bartonella grahamii* DNA was amplified from a bird tick in Korea (10). The substantial mobility, broad distribution, and migrations of birds make them ideal reservoir hosts for dispersal of infectious agents.

To investigate whether birds might be a reservoir for *Bartonella* spp., we screened 86 birds for the presence of *Bartonella* spp. DNA.

The primary study site was a residential backyard in Morehead City, North Carolina, USA (34°43.722'N, 76°43.915'W). Of the 86 birds screened, 78 (16 species) were captured by mist net during March 2010–June 2012 and 8 (3 species) were injured birds that were to be euthanized (Table). Each bird was examined for external abnormalities and ectoparasites, weighed, measured, and tagged with a US Geological Survey–numbered band. A blood sample (0.10–0.25 mL) was collected from each bird by using a 1-mL insulin syringe with a 28-gauge × 1.27-cm needle. Blood remaining after preparation of blood smears was added to an EDTA tube and frozen (–80°C) until processed. Blood smears were examined for hemoparasites. Research was conducted under required state and federal bird banding permits and with the approval of the North Carolina State University Institutional Animal Care and Use Committee.

Before DNA was extracted from the samples, 10 µL of blood was diluted in 190 µL of phosphate-buffered saline. DNA was automatically extracted by using a BioRobot

Symphony Workstation and MagAttract DNA Blood M96 Kit (QIAGEN, Valencia, CA, USA). *Bartonella* DNA was amplified by using conventional *Bartonella* genus PCR primers targeting the 16S–23S intergenic spacer region: oligonucleotides, 425s (5'-CCG GGG AAG GTT TTC CGG TTT ATCC-3') and 1,000as (5'-CTG AGC TAC GGC CCC TAA ATC AGG-3'). Amplification was performed in a 25-mL reaction, as described (3). All PCR reactions were analyzed by 2% agarose gel electrophoresis. Amplicons were sequenced to identify the *Bartonella* sp. and intergenic spacer region genotype. To compare sequences with those in GenBank, we identified bacterial species and genotypes by using Blast version 2.0 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). DNA extraction and PCR-negative controls remained negative throughout the study.

Results are summarized in the Table. None of the screened birds were anemic, but 5 were PCR positive for *Bartonella* spp. (3 for *B. henselae* and 2 for *B. koehlerae*). *B. henselae* was amplified from 2 Northern Mockingbirds (*Mimus polyglottos*) and 1 Red-winged Blackbird (*Agelaius phoeniceus*) (GenBank accession no. KC814161). The DNA sequences were identical to each

other and had 99.6% (456/457 bp) sequence similarity with *B. henselae* San Antonio 2 intergenic spacer region genotype (GenBank accession no. AF369529). *B. koehlerae* was amplified from a Red-bellied Woodpecker (*Melanerpes carolinus*) and a Common Loon (*Gavia immer*) (GenBank accession no. KC814162). The DNA sequences were identical to each other (404/404 bp) and to GenBank sequence AF312490. Lice (Mallophaga order) were found on 5 Boat-tailed Grackles (*Quiscalus major*), but no ectoparasites were observed on *Bartonella* spp.–positive birds. Hemoparasites (*Haemoproteus* and *Plasmodium* spp.) were detected in 7 of 86 birds, indicating exposure to hematophagous ectoparasites, but hemoparasites were not detected in the *Bartonella* spp.–positive birds. No bacteria were visualized in *Bartonella* PCR–positive blood smears.

Bartonella spp. are increasingly associated with animal and human illnesses; thus, the identification of reservoirs and increased understanding of *Bartonella* spp. disease ecology are of public health importance. Our finding of 2 pathogenic species not previously reported in birds has expanded the potential sources for zoonotic infection.

There is growing evidence that migratory birds serve as reservoirs

Table. *Bartonella* species detected in birds*

Bird common name	Bird species	No. birds positive/no. total	<i>Bartonella</i> sp.
House sparrow	<i>Passer domesticus</i>	0/28	
Boat-tailed grackle	<i>Quiscalus major</i>	0/15	
Mourning dove	<i>Zenaidura macroura</i>	0/12	
Herring gull†	<i>Larus argentatus</i>	0/6	
House finch	<i>Carpodacus mexicanus</i>	0/5	
Blue jay	<i>Cyanocitta cristata</i>	0/3	
Song sparrow	<i>Melospiza melodia</i>	0/2	
Northern cardinal	<i>Cardinalis cardinalis</i>	0/2	
Northern mockingbird	<i>Mimus polyglottos</i>	2/2	<i>B. henselae</i> SA2
European starling	<i>Sturnus vulgaris</i>	0/2	
Red-winged blackbird	<i>Agelaius phoeniceus</i>	1/1	<i>B. henselae</i> SA2
Brown thrasher	<i>Toxostoma rufum</i>	0/1	
Tufted titmouse	<i>Baeolophus bicolor</i>	0/1	
Red-bellied woodpecker	<i>Melanerpes carolinus</i>	1/1	<i>B. koehlerae</i>
Common grackle	<i>Quiscalus quiscula</i>	0/1	
Common loon†	<i>Gavia immer</i>	1/1	<i>B. koehlerae</i>
Red-headed woodpecker	<i>Melanerpes erythrocephalus</i>	0/1	
Brown pelican†	<i>Pelicanus occidentalis</i>	0/1	
Collared dove	<i>Streptopelia decacto</i>	0/1	

*SA2, San Antonio 2 intergenic spacer region genotype.

†Euthanized.

and/or mechanical vectors for pathogens such as tick-borne encephalitis virus and *Rickettsia* spp. (6–8). Birds have been implicated as reservoirs for several *Borrelia* spp. (9,10) and for possible dispersion of other tick-borne pathogens (e.g., *Anaplasma* and *Bartonella* spp.) (6,10). Tick transmission of *Bartonella* spp. to birds should be investigated, and additional studies that investigate the reservoir host range of *Bartonella* spp. and the transmission of these bacteria to non-host species will improve epidemiologic understanding of bartonellosis and will identify additional risk factors for *Bartonella* spp. transmission to new hosts, including humans.

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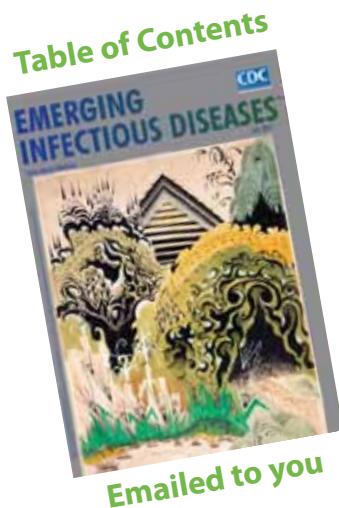
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Tick-borne Pathogens in Northwestern California, USA

To the Editor: In northwestern California, USA, the western black-legged tick, *Ixodes pacificus*, is a known vector of *Borrelia burgdorferi*, the spirochete that causes Lyme disease. *B. miyamotoi*, which is more closely related to spirochetes that cause relapsing fever, has also been detected in 2 locations in California (1,2) and has recently been implicated as a human pathogen in the northeastern United States (3,4). Other studies may have unintentionally included *B. miyamotoi* infections among measures of *B. burgdorferi* if the diagnostics were for spirochetes (e.g., direct fluorescent antibody tests or dark-field microscopy) or genetically targeted for *Borrelia* spp. (5).

To investigate *Borrelia* spp. ecology in California, we collected adult *I. pacificus* ticks by dragging a 1-m² white flannel blanket along vegetation and/or leaf litter in 12 recreational

areas in the San Francisco Bay area during January–May 2012 (Table). Habitat varied from chaparral and grassland to coastal live oak woodland. Ticks were pooled for examination by quantitative PCR (qPCR) for the presence of *Borrelia* spp. We interpreted the prevalence of *Borrelia* spp. from positive pools as the minimum infection prevalence (i.e., assuming 1 positive tick/positive pool). DNA was extracted from ticks by using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocols and then stored at –20°C until use. DNA was analyzed by qPCR, with use of primer and fluorescent hybridization probes previously developed to differentiate *Borrelia* spp. spirochetes (5). To identify the *Borrelia* spp. genotype, we attempted to sequence the 16S–23S (*rrs-rrlA*) intergenic spacer of each sample positive by qPCR (8). The nested PCR product was further purified by using the QIAquick Kit (QIAGEN) and then sequenced (Environmental Genetics and Genomics Laboratory, Northern Arizona University, Flagstaff, AZ, USA; www.enggen.nau.edu/dna.html)

by using capillary Sanger sequencing on an ABI 3730 sequencer (Life Technologies, Grand Island, NY, USA). BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to compare each sequence to other *Borrelia* spp. sequences available from GenBank.

From a total of 1,180 adult ticks, we found 43 samples positive for *Borrelia* spp., resulting in a minimum infection prevalence of 3.6% (Table). We obtained intergenic spacer sequence data for 27 of the positive samples; 6 samples were *B. burgdorferi* sensu stricto, 7 were *B. burgdorferi* sensu lato (both on the basis of alignments of 816 bp), and 14 were *B. miyamotoi* (on the basis of alignments of 503 bp). The *B. miyamotoi* sequences for our samples from California and those for isolates from the eastern United States (9) and Japan (8) formed a monophyletic clade that was oriented as a sister clade to the 3 *Borrelia* spp. that cause tick-borne relapsing fever in the United States (*B. hermsii*, *B. turicatae*, and *B. parkeri*).

We found borreliae-infected adult *I. pacificus* ticks at all 12 sites from which tick sample sizes exceeded 30. When the presence of *B. burgdorferi*

Table. *Borrelia* spp. infection prevalence among adult *Ixodes pacificus* ticks in northwestern California, USA, January–May 2012*

Location, County (reference)	No. <i>Borrelia</i> spp. ticks infected/total (%)				All species
	<i>B. burgdorferi</i> sensu stricto	<i>B. burgdorferi</i> sensu lato	<i>B. miyamotoi</i>	Unsequenced species	
Jasper Ridge Biologic Preserve, San Mateo				1/32 (3.1)	1/32 (3.1)
Pulgas Ridge OSP, San Mateo				2/118 (1.7)	2/118 (1.7)
Thornewood OSP, San Mateo†	1/156 (0.6)	2/156 (1.3)	2/156 (1.3)	4/156 (2.6)	9/156 (5.8)
Thornewood OSP, San Mateo‡					0/9 (0)
Windy Hill OSP, San Mateo†	2/120 (1.7)			1/120 (0.8)	3/120 (2.5)
Windy Hill OSP, San Mateo§	2/122 (1.6)	3/122 (2.5)	1/122 (0.8)	2/122 (1.6)	8/122 (6.6)
Wunderlich County Park, San Mateo					0/15 (0)
Foothills Park, Santa Clara					0/13 (0)
Henry W. Coe State Park, Santa Clara			3/132 (2.3)		3/132 (2.3)
Monte Bello OSP, Santa Clara	1/46 (2.2)			1/46 (2.2)	2/46 (4.3)
Sanborn County Park, Santa Clara			4/53 (7.5)		4/53 (7.5)
Sierra Azul OSP, Santa Clara			2/112 (1.8)		2/112 (1.8)
Los Trancos OSP, San Mateo and Santa Clara			1/58 (1.7)	1/58 (1.7)	2/58 (3.4)
Castle Rock State Park, Santa Cruz		1/51 (2.0)		2/51 (3.9)	3/51 (5.8)
Castle Rock State Park, Santa Cruz (6)					13/264 (4.9)
Tilden Regional Park, Contra Costa (2)	1/814 (0.1)		4/814 (0.5)		5/814 (0.6)
China Camp State Park, Marin		1/143 (0.7)	1/143 (0.7)	2/143 (1.4)	4/143 (2.8)
Hopland Research and Extension Center, Mendocino (1,7)	4/282 (1.4)		2/282 (0.7)		
Total (this study)	6/1,108 (0.5)	7/1,108 (0.6)	14/1,108 (1.3)	16/1,108 (1.4)	43/1,108 (3.6)

*Data are from this study and from previously published research (indicated by reference no.). OSP, open space preserve.

†Woodland.

‡Redwood.

§Chaparral/grassland.