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Bartonella DNA in Loggerhead Sea Turtles

To the Editor: Bartonella are fastidious, aerobic, gram-negative, facultative, intracellular bacteria that infect erythrocytes, erythroblasts, endothelial cells, monocytes, and dendritic cells, and are transmitted by arthropod vectors or by animal scratches or bites (1–6). Currently, 20 species or subspecies of Bartonella have been characterized, of which 8 are known zoonotic pathogens (7). Bartonella henselae has been recently identified from canine blood (8) and from harbor porpoises (9). Pathogenic bacteria are an important threat in terrestrial and marine environments, and in the case of Bartonella henselae, reservoir hosts may be more diverse than currently recognized.

The purpose of this study was to determine whether sea turtles are infected with Bartonella spp. Blood samples were obtained from 29 free-ranging and 8 captive, rehabilitating loggerhead sea turtles (Caretta caretta) from North Carolina coastal waters. Reptilian erythrocytes are nucleated, and commercial lysis methods clogged filtration columns because of the high DNA content of whole blood. Consequently, DNA was extracted from frozen whole blood by using a modified alkaline lysis method adapted from an avian cell culture DNA extraction method (10). PCR screening for Bartonella was performed by using primers for the 16S-23S internal transcribed spacer (ITS) region (Table). Bartonella ITS–positive samples were further screened by using primers for a consensus sequence of the phase-associated gene Pap31 (9). Primers for the 28S rRNA were used as a housekeeping gene. The PCR-positive control contained 0.002 pg/μL of Bartonella H1. Water was the negative PCR control. Amplicons of the expected sizes were consistently obtained from housekeeping gene and positive control reactions, while amplicons were never obtained from negative controls. ITS amplicons were obtained from 16 (43%) of 37 sea turtle blood samples tested, including samples from 13 free-ranging and 3 rehabilitated turtles. Pap31 PCR was performed for Bartonella ITS-PCR–positive samples. Pap31 amplicons were obtained from 5 samples of which 3 were successfully sequenced. Amplification and sequencing of the 16S-23S ITS region detected 2 Bartonella species: a Bartonella-like organisms and 1 more similar to Bartonella vinsonii subsp. berkhoufii. The 3 Pap31 amplicons successfully sequenced confirmed Bartonella henselae infection. Sequences obtained from 1 sample matched Bartonella henselae strains H1-like, the Bartonella henselae SA2-like strain, and Bartonella vinsonii subsp. berkhoufii genotypes II and IV, which suggests that this turtle was co-infected with multiple Bartonella spp. and strains. Three other samples yielded amplicons 99%–100% identical with Bartonella henselae strain SA2, and 3 yielded sequences most similar to Bartonella vinsonii subspecies berkhoufii genotypes II and IV. Two samples contained an ITS region sequence most similar to Bartonella henselae SA2, but with a 15 bp deletion beginning 617 bases downstream of the 16S rRNA gene. Whether these ITS sequence differences represent distinct strains or nonrandom translocation events is uncertain.

Four sea turtle blood samples contained partial ITS sequences most similar to Bartonella vinsonii subsp. berkhoufii. However, these amplicons were much shorter than expected for Bartonella vinsonii subspecies berkhoufii genotype II and genotype IV sequences in GenBank. Because Pap31 gene amplification was unsuccessful for these samples, it is unclear whether small amplicons represent a species related to Bartonella vinsonii subsp. berkhoufii or a new Bartonella sp.

Table. Primers used for PCR amplification

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>28s s</td>
<td>5′-AAACTCTGGTGGAGGTCCGT-3′</td>
</tr>
<tr>
<td>28s as</td>
<td>5′-CTTACCAAAGTGCCCACT-3′</td>
</tr>
<tr>
<td>ITS 325s</td>
<td>5′-CTTCAGATGATGATCCCAAGCTTGG-3′</td>
</tr>
<tr>
<td>ITS 1100as</td>
<td>5′-GACCCGAGACAACCTCGTGGCAAAGCA-3′</td>
</tr>
<tr>
<td>Pap 31 1s</td>
<td>5′-AGTCTGGTTATCCGTTTGGATTTCRRC-3′</td>
</tr>
<tr>
<td>Pap 31 688(as)</td>
<td>5′-AGGACGACAAATGATTCC-3′</td>
</tr>
</tbody>
</table>
To our knowledge, detection of *Bartonella* spp. DNA in sea turtle blood represents the first molecular evidence of *Bartonella* infection in nonmammalian vertebrates. *B. henselae* infection, now reported in porpoises and sea turtles, may represent an emerging infection of marine animals. According to previous studies, immune status appears to affect disease severity, variation in clinical manifestations, the pattern of histopathologic features, and the relative ease of diagnostic detection of the organism (4,7). Although healthy at the time of sample collection, the captive rehabilitated sea turtles were known to have been sick or injured before sampling, potentially reflecting immunocompromise. Whether detection of *Bartonella* spp. in blood of sea turtles is a function of prior immunosuppression induced by stressors is not known. Such stressors could include mechanical injury, malnutrition, environmental toxins, parasites, or concurrent bacterial or viral infections. Alternatively, sea turtles may be a natural marine reservoir for *B. henselae* or for a *Bartonella* sp. genetically related to *B. vinsonii* subsp. *berkhoffii*.

In summary, documentation of *B. henselae* and an organism genetically similar to *B. vinsonii* subsp. *berkhoffii* in the blood of loggerhead sea turtles provides evidence that this genus is not ecologically limited to terrestrial reservoirs. The geographic distribution, prevalence of infection, carrier potential, mode of transmission, and pathogenicity of bloodborne *Bartonella* spp. in sea turtles await additional studies.

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**References**


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**Human Oestrus sp. Infection, Canary Islands**

To the Editor: Myiasis due to *Oestrus ovis* is a well known zoonosis that affects a variety of animals. Human myiasis has also been described and affects mainly persons in rural areas such as shepherds (1) and farmers (2). Although this disease has been reported in both humans and mammals in Spain (3,4), no human case has been described on the Canary Islands. We describe what we believe is the first confirmed case on the islands and discuss the potential utility of serologic diagnosis for this disease.

A 55-year-old farmer from the island of El Hierro, with a medical history of hypercholesterolemia, Q fever, and murine typhus, but currently not being treated, consulted a physician in August 2005 concerning a wormlike sensation in his nose and sinuses that had lasted 2 days. Three days before noticing this sensation, he had been working in his neighbor’s barn, when he noticed that a passing fly “dropped” something in his nose. He also reported sneezing and watery rhinorrhea. These symptoms were self-treated with nasal anticongestants, which provided temporary relief. He finally sought medical attention when a severe cough de-