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***Borrelia miyamotoi* sensu lato in Père David Deer and *Haemaphysalis longicornis* Ticks**

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By sequence analysis of 16S rRNA, *flaB*, *p66*, and *glpQ*, we identified *Borrelia miyamotoi* in 1 of 4 Père David deer ($n = 43$) seropositive for *Borrelia* spp. and 1.2% (3/244) of *Haemaphysalis longicornis* ticks from Dafeng Elk National Natural Reserve, China. Future studies should assess *Borrelia* pathogenesis in deer.

Père David deer (*Elaphurus davidianus*) are extinct in the wild and found only in captivity, principally in China, England, and the United States. Just 5,000 animals remain, with 40% located in Dafeng Elk National Natural Reserve in China, which attracts >1 million tourists annually. Ticks are common in the Dafeng Elk National Natural Reserve (1), so we investigated the tickborne bacterial pathogens in Père David deer at this reserve.

The institutional animal care and use committee of Yangzhou University College of Veterinary Medicine (Yangzhou, China) (YZU-CVM#2015–076) approved this study. We took whole blood samples from 43 apparently healthy Père David deer (20 males, 23 females), separated out the plasma (1,800 × *g* for 10 min), and used the plasma to detect antibodies against bacterial pathogens with the

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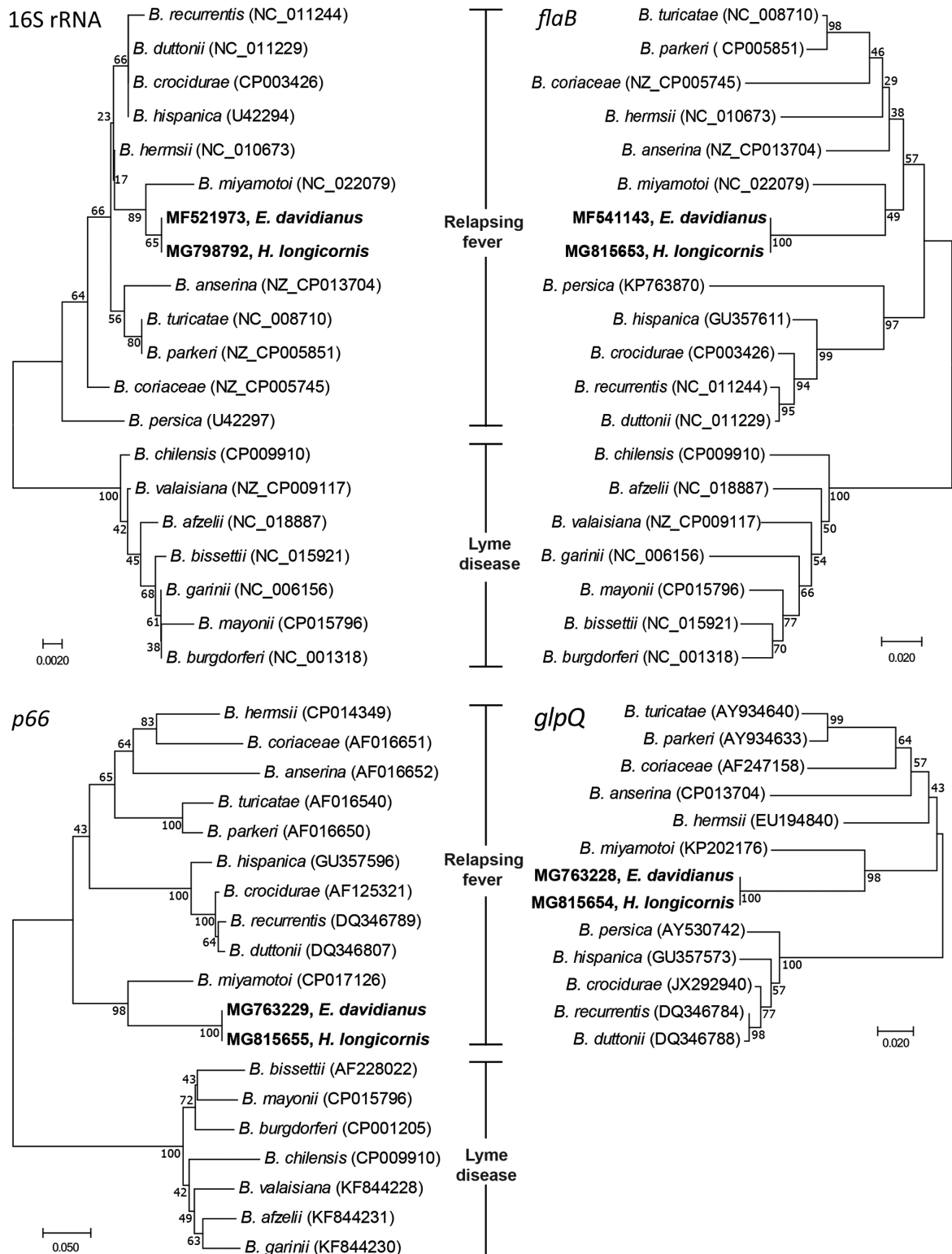


Figure. Neighbor-joining phylogenetic trees constructed with 16S rRNA, *flaB*, *p66*, and *glpQ* gene sequences of *Borrelia* spp. isolates collected from Père David deer (*Elaphurus davidianus*) and *Haemaphysalis longicornis* ticks, Dafeng Elk National Natural Reserve, China, and reference isolates. The isolates identified in this study (bold; GenBank accession nos. MF521973, MF541143, MG763228, MG763229) are most similar to *B. miyamotoi* of the relapsing fever group. Numbers at branch nodes show bootstrap support (1,000 replicates). Scale bars indicate nucleotide substitutions per site.

SNAP 4Dx kit (IDEXX, Westbrook, ME, USA) (2) according to the manufacturer's instructions. Further, ELISAs and Western blots using *Borrelia miyamotoi* GlpQ recombinant protein (RayBiotech, Norcross, GA, USA) and peroxidase-labeled rabbit anti-deer IgG (SeraCare, Milford, MA, USA) were performed as described previously (3) to detect GlpQ antibodies specific to *B. miyamotoi*.

We collected a convenience sample of *Haemaphysalis longicornis* ticks (n = 244) from elk in the Dafeng Elk National Natural Reserve during the summer of 2016 and stored the collection at -80°C . We used the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions to extract DNA from Père David deer whole blood samples and entire *H. longicornis* ticks. We used published PCR protocols targeting the 16S rRNA (4), *flaB* (5), and *glpQ* (6) genes and an in-house *p66* PCR (forward primer 5'-CGATTTTCTATATTGGACACAT-3', reverse primer 5'-GATATAGATTCTACAGGTATTGCATAATC-3') to screen blood samples and ticks for *B. miyamotoi*. We sequenced both strands of PCR products using BGI's (Shanghai, China) services and aligned them using ClustalW in MEGA 7 (<http://www.megasoftware.net/>) with the nucleotide sequences of 11 relapsing fever group borreliae and 7 Lyme disease group borreliae found in GenBank.

Four (9.3%; 1 male, 3 females) of the 43 deer were seropositive by SNAP 4Dx, demonstrating an immunodominance of antibodies against synthetic C6 peptide invariable region 6 of the pathogenic *Borrelia* genospecies, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* (7). Seropositivity was confirmed by GlpQ antibody ELISA and Western blot with GlpQ recombinant protein, indicating exposure to *B. miyamotoi*.

One of the seropositive female deer (2.3% of overall deer population) and 3 (1.2%) of the 244 ticks were positive for the 4 *Borrelia* genes tested (16S rRNA, *flaB*, *glpQ*, *p66*) by PCR. The sequences obtained from the PCR products showed the 4 animals had identical sets of *Borrelia* genes. The 16S rRNA, *flaB*, and *p66* sequences were more similar to those of the relapsing fever group borreliae (16S rRNA 97.9%–99.3%, *flaB* 83.7%–88.9%, *p66* 72.4%–83.3%) than the Lyme disease group borreliae (16S rRNA 96.6%–97.2%, *flaB* 79.2%–80.9%, *p66* 66.1%–68.1%). The *B. miyamotoi glpQ* gene sequence obtained from the deer and ticks also clustered with those of the relapsing fever group borreliae (81.1%–88.9%), and all analyzed gene sequences had greatest similarity with *B. miyamotoi* genes (16S rRNA 99.3% [576/580], *flaB* 88.9% [321/361], *p66* 83.3% [423/508], *glpQ* 88.9% [377/424]) (Figure).

B. miyamotoi is a member of the relapsing fever group first isolated in Japan and subsequently found in North America, Europe, and Russia (8). *B. miyamotoi* has not

been reported in deer but can be pathogenic in humans, usually resulting in an acute febrile influenza-like illness but occasionally causing severe disease, including meningoencephalitis (9). Further studies are needed to determine the effects of *B. miyamotoi* infections in deer, especially because studies on *Ixodes scapularis* ticks in the United States have indicated that deer might be a sylvatic reservoir (10).

I. persulcatus and *I. pavlovskyi* ticks are known to be infected with *B. miyamotoi* in Asia, whereas other *Ixodes* spp. ticks are vectors in the United States and Europe (9). Tick control in semi-free-ranging animals is challenging; the Père David deer we studied are commonly infested with ticks. The only tick species identified on Père David deer in Dafeng Elk National Natural Reserve was *H. longicornis* (1), which can reach high densities in the environment (summer 89.5 ± 17.1 ticks/10 m², winter 1.47 ± 0.35 ticks/10 m²) and cause anemia and even death in heavily infested animals. Our finding of *B. miyamotoi* in *H. longicornis* ticks adds to the list of organisms reported in this tick, primarily *B. burgdorferi* sensu lato and unclassified *Borrelia* spp.

In summary, we have shown that *B. miyamotoi* sensu lato occurs in Père David deer and *H. longicornis* ticks in Dafeng Elk National Natural Reserve. Further studies are needed on the pathogenicity of the organism in deer and the role of *H. longicornis* ticks in the epidemiology of infections in deer and humans.

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***Rickettsia asemonensis* Characterization by Multilocus Sequence Typing of Complete Genes, Peru**

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While studying rickettsial infections in Peru, we detected *Rickettsia asemonensis* in fleas from domestic animals. We characterized 5 complete genomic regions (17kDa, *gltA*, *ompA*, *ompB*, and *sca4*) and conducted multilocus sequence typing and phylogenetic analyses. The molecular isolate from Peru is distinct from the original *R. asemonensis* strain from Kenya.

Rickettsia asemonensis belongs to a group of *R. felis*-like organisms (RFLOs) that are similar, yet distinct, from their closest known relative, *R. felis* (1,2). Although *R. felis* causes disease in humans (3), the pathogenicity of RFLOs remains unknown (1,4,5). *R. asemonensis* was initially identified in domestic fleas from Kenya (1). Subsequently, reports from the Americas, Asia, and Africa established that *R. asemonensis* is ubiquitous and closely associated with human habitats because of its arthropod hosts (4–7). However, reports of *R. asemonensis* rarely include robust genomic information needed to establish degrees of genetic diversity. Consequently, many rickettsial infections remain underdiagnosed, even when prevalence is high (8). We recently described *R. asemonensis* in multiple ectoparasites (*Ctenocephalides felis* fleas and *Rhipicephalus sanguineus* ticks) collected in the Peruvian Amazon (9). Here, we detail multilocus sequence typing of a single molecular isolate using next-generation sequencing data for 5 complete genomic regions, including conserved (17kDa and *gltA*) and variable (*ompA*, *ompB*, and *sca4*) genes.

The internal review board of the US Naval Medical Research Unit No. 6 and the Institutional Animal Care and Use Committee approved the study protocol in compliance with all applicable regulations. Genomic DNA was mechanically extracted from half of a single *C. felis* flea as described (9) and fragmented by Bioruptor (Diagenode, Denville, NJ, USA). Fragmented DNA served as template to prepare IonPGM libraries using IonPlus Fragment Library Kits (ThermoFisher, Lima, Peru) according to the manufacturer's directions. We conducted quality control using Bioanalyzer High Sensitivity chips (Agilent, Lima, Peru). We prepared libraries for sequencing using IonPGM Template OT2 200 Kits (ThermoFisher, Lima, Peru) and conducted sequencing on 318 chips using IonPGM Sequencing 200 Kits v2 (ThermoFisher). We processed raw data by reference mapping against NMRCii from Kenya (10). Of the 20,575,878 shotgun sequencing reads generated, ~12% matched Rickettsiaceae.

Comparison of the consensus sequences we generated (GenBank accession nos. KY650696–KY650700) with those of strain NMRCii (GenBank accession no.