

found suitable for the diagnosis of OROV infections in the acute phase. Thus, a combination of a systematic surveillance for acute febrile illnesses and efficient laboratory diagnosis for OROV resulted in the discovery of an outbreak, which would probably have been overlooked if it had occurred in any region simultaneously with large dengue outbreaks or in the absence of laboratory diagnosis. The cases of OROV fever reported here likely represent a small portion of the cases; a much higher number of cases probably occurred in Manaus during the study period.

The clinical characteristics of most cases of OROV fever in this outbreak were similar to previously reported descriptions of the illness. Notably, however, 20 (15.5%) patients from Manaus had spontaneous hemorrhagic phenomena (petechiae, epistaxis, and gingival bleeding) that had not previously been described in OROV fever (4–6). Moreover, symptoms of involvement of the central nervous system were not observed.

In recent years, the area of circulation and the epidemic potential of OROV have increased, and this virus has emerged as a public health problem in Brazil and other countries in the Americas. Presently, OROV is the most common of the Brazilian zoonotic arboviruses infecting humans (7). Further evidence of the spread of OROV was its isolation in 2003 from a small primate, a marmoset (*Calithrix*), in the state of Minas Gerais in southeast Brazil, far from the Amazon region (8). Considering that midges (*Culicoides paraensis*) occur in most low altitude areas of the Americas, it is conceivable that environmental destruction and climate changes could result in OROV outbreaks in the large cities of Brazil, as well as in other parts of the Western Hemisphere (9).

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**Maria Paula G. Mourão,
Michelle S. Bastos,
João Bosco L. Gimaque,
Bruno Rafaelle Mota,
Giselle S. Souza,
Gustavo Henrique N. Grimmer,
Elizabeth S. Galusso,
Eurico Arruda,
and Luiz Tadeu M. Figueiredo**

Author affiliations: Tropical Medicine Foundation of Amazonas State, Manaus, Brazil (M.P.G. Mourão, M.S. Bastos, J.B.L. Gimaque, E.S. Galusso); Amazonas State University, Manaus (M.P.G. Mourão, J.B.L. Gimaque, B.R. Mota); Nilton Lins University Center, Manaus (M.P.G. Mourão, E.S. Galusso); Leonidas and Maria Deane Research Center, Manaus (G.S. Souza, G.H.N. Grimmer); and University of São Paulo School of Medicine, Ribeirão Preto, Brazil (E. Arruda, L.T.M. Figueiredo)

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Address for correspondence: Maria Paula G. Mourão, Tropical Medicine Foundation of Amazonas (Laboratory of Virology), Av Pedro Teixeira, 25, Manaus, Amazonas, Brazil 69040-000; email: mpmourao@uol.com.br

Identical Strains of *Borrelia hermsii* in Mammal and Bird

To the Editor: On August 5, 1994, a northern spotted owl, *Strix occidentalis caurina*, was found dead in Kittitas County, Washington, USA (1). A thorough investigation and necropsy identified the probable cause of death to be a spirochete infection. The organisms were seen in sections of the bird's liver with use of modified Steiner silver stain and microscopy. DNA was extracted from the infected liver, and PCR–DNA sequencing of the 16S ribosomal RNA (rRNA) locus identified the bacterium as a relapsing fever spirochete related most closely to *Borrelia hermsii* (1). The lack of additional data surrounding this case precluded Thomas et al. from concluding that this spirochete

infecting the owl was *B. hermsii* (1). Yet, in a subsequent analysis using the intergenic spacer region, the owl spirochete was included with isolates of *B. hermsii* (2).

To investigate the distribution and prevalence of *B. hermsii*, during the summer of 2008, we began a study at Flathead Lake, Lake County, Montana, USA, where 9 persons have contracted relapsing fever since 2002 (3–5). A blood smear from 1 pine squirrel (*Tamiasciurus hudsonicus*) captured July 9 at Yellow Bay on the east shore of the lake (elevation 887 m; geographic coordinates 47°52'35"N, 114°01'54"W) contained spirochetes detected when stained with Giemsa and examined by microscopy (600× brightfield with oil immersion). Whole blood from the squirrel contained live spirochetes visible by dark-field microscopy, and ≈50 μL of this blood was injected intraperitoneally into a laboratory mouse. The next day, a few spirochetes were observed in the peripheral blood of the mouse, and during the next 3 days, the density of spirochetes increased. We used intracardiac puncture to collect blood from the mouse for spirochete isolation in BSK-H medium (Sigma-Aldrich, St Louis, MO, USA) and for analysis by PCR and DNA sequencing of multiple bacterial loci as described elsewhere (4,6).

The spirochetes observed in the squirrel's blood failed to grow in

BSK-H medium after passage in the laboratory mouse; however, we acquired DNA sequences from infected squirrel and mouse blood from PCR amplicons for 6 spirochete loci including *16S rDNA*, *flaB*, *gyrB*, *glpQ*, IGS, and *vtg*. Sequences for the loci were each aligned with homologous sequences from other borrelia in our collection, and each locus grouped the spirochete within the 2 genomic groups of *B. hermsii* described previously (4,6). The unique squirrel spirochete differed from all other *B. hermsii* identified in our previous studies; deep branches in each phylogram grouped the spirochete more closely with *B. hermsii* genomic group I than with genomic group II (data not shown).

Next, we compared the sequences from the squirrel spirochete with those available in the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov), including those sequences reported for the spirochete found in the spotted owl (AY515269.1, AF116903.1, AF116904.1) (1,2). The 3 trimmed and aligned sequences for the *16S rDNA* (1,290 bases), *flaB* (467 bases), and IGS (665 bases) from the squirrel spirochete were identical to those of the owl spirochete; no base differences were found among the 2,422 bases compared. We also examined DNA extracted from the spotted owl's liver during the first in-

vestigation (1) (provided by Alan G. Barbour). We successfully PCR amplified most of the *16S rDNA* and the complete *flaB*, *gyrB*, *glpQ*, and *vtg* genes from the owl spirochete DNA and determined their sequences. The complete sequences of the first 4 loci from the owl and squirrel spirochetes were identical and differed from all other *B. hermsii* sequences. A phylogram of the concatenated sequences totaling 5,188 bases demonstrated that the owl and pine squirrel spirochetes were identical and were divergent members of *B. hermsii* genomic group I (Figure).

Finding the same strain of *B. hermsii*, separated by ≈525 km, in a pine squirrel and a spotted owl demonstrates a broader geographic distribution and host range for this spirochete than what could have been envisaged previously. The possible role of birds as hosts for the vector *Ornithodoros hermsi* ticks has been demonstrated elsewhere (4). Given the ecologic overlap of pine squirrels and coniferous forest-dwelling birds, we believe that the previous finding of the infected spotted owl is likely not an isolated event. Instead, it may represent a tick-spirochete cycle for *B. hermsii* that includes a broader host range for this group of relapsing fever spirochetes than previously appreciated.

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**Robert J. Fischer,
Tammi L. Johnson,
Sandra J. Raffel,
and Tom G. Schwan**

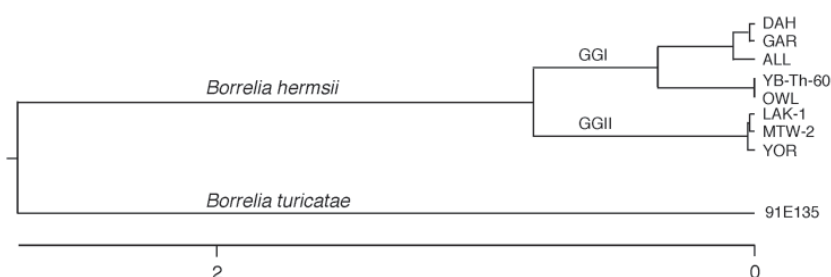


Figure. Phylogram based on the alignment of the concatenated DNA sequences containing the *16S rDNA*, *flaB*, *gyrB*, and *glpQ* loci for 6 isolates (DAH, GAR, ALL, LAK-1, MTW-2, and YOR) and infected tissues from the owl (OWL) and pine squirrel (YB-Th-60) of *Borrelia hermsii*. The same loci from *B. turicatae* 91E135 were used for the outgroup. New DNA sequences determined for the owl and pine squirrel spirochetes are available in GenBank (accession nos. GQ175059–GQ175068). Scale bar indicates number of base substitutions (×100).

Author affiliations: National Institutes of Health, Hamilton, Montana, USA (R.J. Fischer, T.L. Johnson, S.J. Raffel, T.G. Schwan); and The University of Montana, Missoula, Montana, USA (T.L. Johnson)

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Address for correspondence: Tom G. Schwan, Laboratory of Zoonotic Pathogens, Rocky Mountain Laboratories, NIAID, NIH, 903 South 4th St, Hamilton, MT 59840, USA; e-mail: tschwan@niaid.nih.gov

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Mycobacterium bovis and *M. tuberculosis* in Goats, Nigeria

To the Editor: Documentation of possible tuberculosis (TB) in goats in Nigeria was reported by Ojo (1) on the basis of gross lesions without culture confirmation. Livestock owners in Nigeria normally graze cattle and goats together, and this practice poses a high risk for transmission of bovine TB among these animals (1). This practice is especially a threat to goats in Nigeria because several reports have described bovine TB in cattle in Nigeria (2–5). However, reports of diagnosis of TB in goats in Nigeria are lacking.

Molecular epidemiologic techniques such as deletion typing and spoligotyping have been used to characterize members of the *Mycobacterium tuberculosis* complex (MTC) and to provide information on transmission of mycobacterial diseases between animals and humans (6). However, because of limited resources and lack of expertise, these techniques are not commonly used in most developing nations such as Nigeria, where TB is endemic (3).

Because slaughterhouses provide excellent opportunities for detecting diseases of economic and public health importance, we investigated the presence of mycobacteria in slaughtered goats with lesions suggestive of TB. The investigation was conducted at the Bodija Municipal Abattoir in Ibadan in southwestern Nigeria over a period of 6 months. Slaughtered goats were obtained from local herds and herds in northern Nigeria. A total of 10,389 male and female goats of 2 breeds (West African Dwarf and Red Sokoto) and 1–2 years of age were slaughtered; 1,387 were inspected for gross lesions of TB.

Of 1,387 animals screened, 62 (4.47%) had lesions suggestive of

TB in the liver, lungs, and mesenteric lymph nodes. Five (0.36%) goats were confirmed positive by culture as described (2). Deletion typing (6) with the RD9 deletion was used to distinguish *M. tuberculosis* from other members of the MTC. Those isolates with a deletion in this region were further investigated with primers specific for RD4. This reaction distinguishes between *M. bovis*, *M. caprae*, and other members of the MTC. Spoligotyping was performed as described (7) to type an *M. tuberculosis* isolate from a goat after identification of this bacterium by deletion typing.

We isolated 4 strains of *M. bovis* and 1 strain of *M. tuberculosis* from the goats (Table). Spoligotyping identified the *M. tuberculosis* isolate as belonging to the East African Indian (EAI)–5 family in the SpolDB4 database. All *M. bovis* isolates were *M. bovis bovis*, not *M. bovis caprae*, according to their deletion typing profile (6). One *M. bovis* isolate was obtained from a male goat; the 3 remaining *M. bovis* isolates and the *M. tuberculosis* isolate were obtained from female goats.

Epidemiologic inferences can be made from the results of our study. First, *M. bovis*, which is naturally found in cattle, was isolated from 4 slaughtered goats. Although *M. bovis caprae* was the *M. bovis* variant most frequently isolated from goats in some areas (8), in our study, only *M. bovis bovis* was isolated. This finding is consistent with results reported by Crawshaw et al. (9), and suggests transmission from cattle, rather than transmission from the goat reservoir. Second, because the infected goats were adult females, infection may be transmitted to their offspring. Third, *M. tuberculosis* was isolated from a goat. Its presence in this goat may have been caused by direct transmission from humans because this bacterium may be a natural pathogen of humans.

Transmission caused by close cohabitation of goats and humans