

Leptospira noguchii and Human and Animal Leptospirosis, Southern Brazil

To the Editor: Pathogenic leptospires, the causative agents of leptospirosis, exhibit wide phenotypic and genotypic variations. They are currently classified into 17 species and >200 serovars (1,2). Most reported cases of leptospirosis in Brazil are of urban origin and caused by *Leptospira interrogans* (3). Brazil underwent a dramatic demographic transformation due to uncontrolled growth of urban centers during the last 60 years. Urban slums are sites of poor sanitation that favors rat-borne transmission of leptospirosis among humans. Thus, this may explain the major involvement of serovar Copenhageni (*L. interrogans*). The predominance of *L. interrogans* is likely due to the underestimation of rural cases of leptospirosis.

Pelotas is a coastal city in Rio Grande do Sul State, in southern Brazil, with ≈400,000 inhabitants. This state has a typical temperate climate. However, the incidence of human leptospirosis is high (12.5/100,000 inhabitants in 2001) compared with the mean incidence in areas of Brazil where tropical and subtropical climates predominate (3.5/100,000 in the same year). Most cases in Rio Grande do Sul State (69%) occur in rural areas where the spatial distribution suggests an association with areas of rice field activities. Pelotas had an annual incidence of >50 cases per 100,000 inhabitants in 2001, which placed it among the cities with the highest incidence of leptospirosis in southern Brazil (4).

Before 2007, pathogenic serovars and strains in Brazilian collections included the following species: *L. santarosai*, *L. interrogans*, *L. kirshneri*, and *L. borgpetersenii*. However, our research group has recently reported the isolation of *L. noguchii* in Brazil from sheep (5). This species had been

previously isolated from animals such as armadillo, toad, spiny rat, opossum, nutria, the least weasel (*Mustela nivalis*), cattle, and the oriental fire-bellied toad (*Bombina orientalis*) in Argentina, Peru, Panama, Barbados, Nicaragua, and the United States (1,6). Human leptospirosis associated with *L. noguchii* has been reported only in the United States, Peru, and Panama, with the isolation of strains Autumnalis Fort Bragg, Tarassovi Bac 1376, and Undesignated 2050, respectively (1,6). The Fort Bragg strain was isolated during an outbreak among troops at Fort Bragg, North Carolina. It was identified as the causative agent of an illness characterized by fever, headache, myalgia, and a pretibial rash—Fort Bragg fever (7). We were not able to obtain data regarding the other 2 human isolates.

We report the isolation of 3 additional *L. noguchii* strains from Brazil, including 2 from cases of human leptospirosis. The first isolate (Bonito strain) was obtained from the blood culture of a 34-year-old man who exhibited fever, headache, myalgia, hemorrhages, jaundice, abdominal pain, diarrhea, and vomiting. The patient reported contact with rats, farm animals, and dogs before the onset of illness. Laboratory tests at admission to the Hospital Santa Casa de Misericórdia, Pelotas, showed an elevated level of serum bilirubin (total 21 mg/dL, direct 16 mg/dL) and a slight increase in liver enzyme levels (alanine aminotransferase 2×, aspartate aminotransferase 1.5× above reference levels). An acute-phase serum sample showed a titer of 25 against serovars Autumnalis and Bratislava by microscopic agglutination test (MAT).

The second isolate (Cascata strain) was obtained from the blood culture of a 16-year-old boy who exhibited headache, fever, flulike symptoms, and myalgia. He reported previous contact with rats and dogs. The patient was not hospitalized, and an acute-phase serum sample showed a

titer of 25 against saprophytic serovar Andamana by MAT. Both patients were from the rural area of Pelotas. Unfortunately, convalescent-phase serum samples were not obtained from these patients.

A third isolate (Hook strain) was obtained from a male stray dog with anorexia, lethargy, weight loss, disorientation, diarrhea, and vomiting. The animal died as a consequence of the disease. The isolate was obtained from a kidney tissue culture. No temporal or spatial relationship was found between the 3 cases.

Serogrouping was performed by using a panel of rabbit antisera. Bonito, Cascata, and Hook strains were classified as Autumnalis, Bataviae, and Australis, respectively. Serogroups were confirmed by the strong and specific reaction of hyperimmune sera against these isolates, with the reference strains of the respective serogroups. Species identification was accomplished by sequencing nearly the full length of the 16S rRNA gene, as previously described (5). The sequences of the Hook, Cascata, and Bonito strains were deposited in GenBank under accession nos. EU349494–EU349496.

In addition, the *rpoB* gene sequence was determined and used for further confirmation of the species. The *rpoB* sequence for the strains Hook, Cascata, Bonito, and the *L. noguchii* reference strains were deposited in GenBank under accession nos. EU349497–EU349505. BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) alignment confirmed the new isolates as *L. noguchii*. The 16S rRNA gene sequence was also used for taxonomic analysis of *L. noguchii* (Figure). The topology-based dendrogram demonstrates sequence relatedness among strains isolated in Pelotas and the *L. noguchii* Autumnalis, Australis, and Bataviae strains deposited in GenBank (Figure). No molecular or serologic characterization at the serovar level was performed.

We report herein the occurrence of *L. noguchii* species in southern Brazil. The 3 isolates obtained belong to distinct serogroups. Information presented here places *L. noguchii* among the prevalent *Leptospira* species that are able to cause human and animal leptospirosis in southern Brazil.

Isolation procedures and DNA sequencing were conducted at the Federal University of Pelotas. Serogrouping of

the isolates was performed at the Gonçalo Moniz Research Centre.

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References

- Brenner DJ, Kaufmann AF, Sulzer KR, Steigerwalt AG, Rogers FC, Weyant RS. Further determination of DNA relatedness between serogroups and serovars in the family *Leptospiraceae* with a proposal for *Leptospira alexanderi* sp. nov. and four new *Leptospira* genospecies. *Int J Syst Bacteriol.* 1999;49:839–58.
- Salaun L, Merien F, Gurianova S, Baranton G, Picardeau M. Application of multilocus variable-number tandem-repeat analysis for molecular typing of the agent of leptospirosis. *J Clin Microbiol.* 2006;44:3954–62. DOI: 10.1128/JCM.00336-06
- Pereira MM, Matsuo MG, Bauab AR, Vasconcelos SA, Moraes ZM, Baranton G, et al. A clonal subpopulation of *Leptospira interrogans* sensu stricto is the major cause of leptospirosis outbreaks in Brazil. *J Clin Microbiol.* 2000;38:450–2.
- Barcellos C, Lammerhirt CB, de Almeida MA, Santos E. Spatial distribution of leptospirosis in Rio Grande do Sul, Brazil: recovering the ecology of ecological studies [in Portuguese]. *Cad Saude Publica.* 2003;19:1283–92. DOI: 10.1590/S0102-311X2003000500007
- Silva EF, Brod CS, Cerqueira GM, Bourscheidt D, Seyffert N, Queiroz A, et al. Isolation of *Leptospira noguchii* from sheep. *Vet Microbiol.* 2007;121:144–9. DOI: 10.1016/j.vetmic.2006.11.010
- Faine SB, Adler B, Bolin C, Perolat P. *Leptospira* and leptospirosis, 2nd ed. Melbourne (Australia): MediSci; 1999.

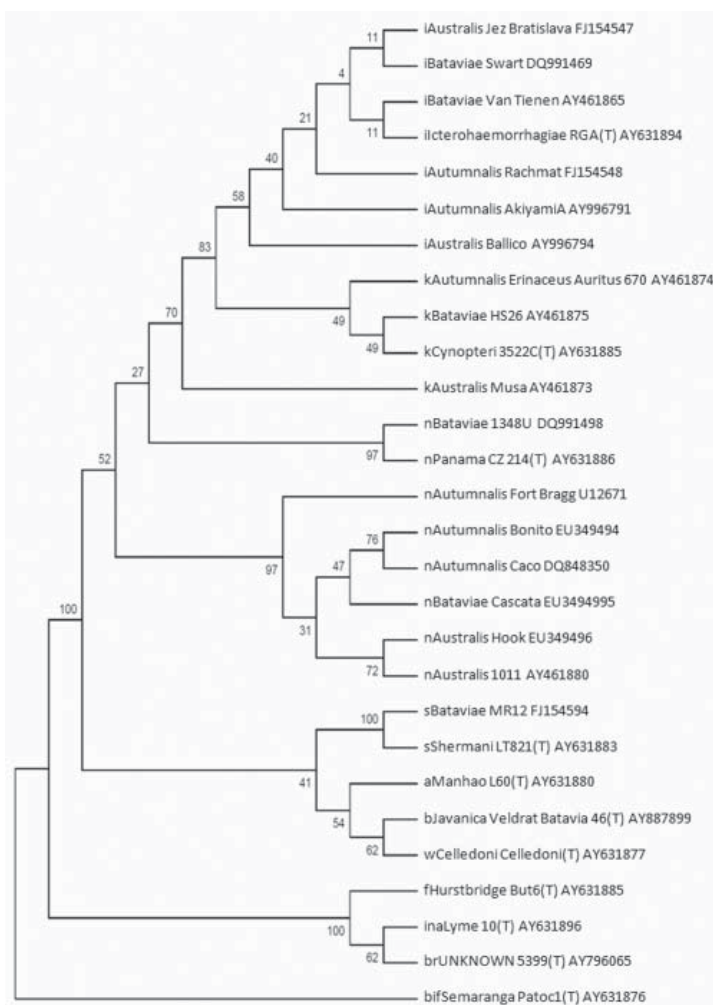


Figure. Dendrogram constructed by using the neighbor-joining algorithm, based on a 1,180-bp sequence of the 16S rRNA gene demonstrating the position of the Brazilian strains (Bonito, Cascata, and Hook) within the *Leptospira noguchii* species. This dendrogram summarizes, by bootstrap-based topology, the evolutionary relationship among *L. noguchii* strains. The bootstrap consensus values are indicated over each root. The initial lowercase letters indicate the respective species to which each strain belongs: i, *L. interrogans*; k, *L. kirschneri*; n, *L. noguchii*; b, *L. borgpetersenii*; w, *L. weilii*; s, *L. santarosai*; a, *L. alexanderi*; f, *L. fainei*; in, *L. inadai*; br, *L. broomi*; bif, *L. biflexa*. (T) indicates the type-strain for each species. The GenBank accession number follows the strain identification.

7. Fraser DW, Glosser JW, Francis DP, Phillips CJ, Feeley JC, Sulzer CR. Lep-tospirosis caused by serotype Fort-Bragg. A suburban outbreak. *Ann Intern Med.* 1973;79:786–9.

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Aquaculture and Florfenicol Resistance in *Salmonella enterica* Serovar Typhimurium DT104

To the Editor: In a letter recently published in *Emerging Infectious Diseases*, Smith (1) discussed evidence that he mistakenly believes to undermine the hypothesis that the florfenicol resistance gene present in some isolates of the epidemic *Salmonella enterica* serovar Typhimurium DT104 strain originated from a florfenicol resistance plasmid present in *Vibrio damsela* (*Pasteurella piscicida*) that infected fish farms in Japan in the 1990s (2). Smith correctly states that the florfenicol resistance gene was present in *S. enterica* serovar Typhimurium DT104 strains isolated in the United States in 1985, before the gene was documented in *V. damsela* in Japan (1,3). He is also correct in noting that this particular florfenicol resistance gene was detected in a plasmid in *Klebsiella pneumoniae* in France in 1969 (1,4).

However, an earlier report by Briggs and Fratamico (5) clearly established that the florfenicol resistance genes and the tetracycline resistance genes *tetG* and *tetR* in the *Salmonella*

genomic island 1 (SGI1) were surrounded by non-antimicrobial-drug resistance DNA. This DNA is homologous to DNA sequences in plasmids PASPPFLO and pJA8122 (see Figure 1 and Table 2 in reference 5) (5–7). In addition to antimicrobial drug resistance genes, PASPPFLO and pJA8122 contain cloned DNA segments of indigenous R plasmids found in *V. damsela* and *V. anguillarum*, respectively; these cloned DNA segments span sequences that extend beyond their florfenicol resistance and *tetR/tetG* genes (5–7). For example, the region of the florfenicol resistance gene in SGI1 contains 763 nt of the non-antimicrobial-drug resistance portion of the original *V. damsela* plasmid; the region of *tetR/tetG* contains 468 nt of the non-antimicrobial-drug resistance DNA segment of the *P. piscicida* plasmid (5–7).

The presence of these non-antimicrobial-drug resistance R plasmid DNA sequences in SGI1 constitutes a molecular signature that firmly establishes the aquaculture origin of the florfenicol resistance and the *tetR/tetG* genes in the *S. enterica* serovar Typhimurium DT104 strain studied by Briggs and Fratamico and in the SGI1 of other bacteria (5). These R plasmid DNA sequences in SGI1 also confirm direct or indirect horizontal gene transfer between bacteria in the aquaculture environment and *S. enterica* serovar Typhimurium DT104 (5–7).

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References

- Smith P. Aquaculture and florfenicol resistance in *Salmonella enterica* Typhimurium DT104. *Emerg Infect Dis.* 2008;14:1327–8. DOI: 10.3201/eid1412.080162
- Angulo FJ, Griffin PM. Changes in antimicrobial resistance in *Salmonella enterica* serovar Typhimurium. *Emerg Infect Dis.* 2000;6:436–8.
- Ribot EM, Wierzbza RK, Angulo FJ, Barrett TJ. *Salmonella enterica* serotype Typhimurium DT104 isolated from humans, United States, 1985, 1990, and 1995. *Emerg Infect Dis.* 2002;8:387–91.
- CloECKaert A, Baucheron S, Chalus-Dancla E. Nonenzymatic chloramphenicol resistance mediated by IncC plasmid R55 is encoded by a *floR* gene variant. *Antimicrob Agents Chemother.* 2001;45:2381–2. DOI: 10.1128/AAC.45.8.2381-2382.2001
- Briggs CE, Fratamico PM. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. *Antimicrob Agents Chemother.* 1999;43:846–9.
- Kim E, Aoki T. Sequence analysis of the florfenicol resistance gene encoded in the transferable R-plasmid of a fish pathogen, *Pasteurella piscicida*. *Microbiol Immunol.* 1996;40:665–9.
- Zhao J, Aoki T. Nucleotide sequence analysis of the class G tetracycline resistance determinant from *Vibrio anguillarum*. *Microbiol Immunol.* 1992;36:1051–60.

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In Response: In his letter (1), Cabello makes 2 observations regarding the debate concerning the origin of the *floR* gene in *Salmonella enterica* serovar Typhimurium DT104. The first observation is that the plasmid PASPPFLO contained cloned segments of an indigenous *Vibrio damsela* plasmid. However, PASPPFLO is not the name of a plasmid but is the GenBank locus identifier associated with the sequence (GenBank accession no. D37826) of a 3,745-bp region of the *V. damsela* plasmid pSP92088 that contained pp-*flo* (2,3).

The second observation is that sequences flanking the *floR* gene in *S. enterica* serovar Typhimurium DT104 (GenBank accession no. AF071555) are homologous to those flanking the pp-*flo* gene sequenced from the *V. damsela* plasmid pSP92088 (4). On the basis of this homology, he seems