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Short Communication

Molecular and serological characterization of *Leptospira kirschneri* serogroup Pomona isolated from a human case in a Brazilian rural area

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Abstract

Introduction: Leptospirosis is an important health concern in Brazil. Currently, information on the epidemiology of the disease in the rural areas of the country is lacking. **Methods:** Serological and molecular techniques were used to characterize a clinical isolate of *Leptospira*. **Results:** The strain CLEP 00060, isolated from a 59-year-old man in a rural area of Rio Grande do Sul state, Brazil, was identified as belonging to *L. kirschneri* serogroup Pomona serovar Mozdok. **Conclusions:** This study contributes to the local epidemiological knowledge of leptospirosis, prevention of the disease by vaccines, and improvements in its diagnosis.

Keywords: *Leptospira kirschneri.* Leptospirosis. Typing.

Leptospirosis is a worldwide zoonosis, being more common in tropical regions and predominantly found in impoverished populations inhabiting developing countries, where incidence peaks are observed during the raining season[1]. Although leptospirosis is currently recognized as a disease of epidemic potential with a significant impact on public health in many countries, it remains neglected[2]. This can be explained by its common incidence in areas where socioeconomic (poverty, lack of water and sanitation, poor housing conditions) and environmental (heavy rains or floods) factors are crucial for the maintenance of leptospires and subsequent occurrence of the disease[2,3].

Although considered the definitive diagnosis for leptospirosis, the isolation of *Leptospira* is not a timely aid for the clinical care of human patients, being a method rarely achieved[4]. Moreover, the identification of clinical isolates at the serovar level is a laborious process and is restricted to a few reference laboratories[1].

Despite these challenges, identifying the infective serovar is very important for diagnosis and epidemiological studies, because it can provide information related to the host reservoirs involved in pathogen transmission and, therefore, contribute to the adoption of multidisciplinary control strategies[2-4].

Concerning the epidemiology of leptospirosis, there is a lack of information on the countryside areas of Brazil, compared to that on the urban areas where serovars Copenhageni and Icterohaemorrhagiae are predominant[2,5]. However, it should be highlighted that there are reports of other serovars found in different species of domestic and wild animals in those areas[6,7].

Leptospirosis is an important public health problem in the State of Rio Grande do Sul (Southern Brazil), with an average of 428 cases reported annually. A study conducted using the One Health approach showed that rural populations of the state have an approximately eight times higher risk of contracting leptospirosis than their urban counterparts do, even though the number of reported cases was high in both areas[2].

In the present study, we demonstrate the identification of a clinical isolate obtained from a human anicteric case in a rural area of Southern Brazil.

A 59-year-old male worker from a rice-planting farm in Southern Brazil (Santa Vitória do Palmar City, Rio Grande do Sul State), presenting with acute fever, myalgia, malaise, headache, vomiting, and diarrhea, had his blood and serum collected on the fifth and ninth day of symptoms, respectively. Serological tests immunoglobulin M-enzyme-linked immunosorbent assay (IgM-ELISA) (Bio-Manguinhos/FIOCRUZ) and microscopic agglutination test (MAT)[8] — showed positive results for *Leptospira* spp. The reactive serogroups and their respective

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titers obtained with MAT were as follows: Icterohaemorrhagiae, 400; Australis, 200; and Pomona, 100.

Blood culture was positive for the presence of *Leptospira*, visualized using dark field microscopy[8]. The culture was purified, maintained by subculturing in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium[8], and stored at the Collection of Leptospira [(CLEP) Oswaldo Cruz Institute/ FIOCRUZ)] under the registration number CLEP 00060.

Microscopic agglutination test was also carried out against reference antisera (obtained from the Royal Tropical Institute, Netherlands) representative of the 15 most prevalent serogroups in Brazil: Icterohaemorrhagiae, Canicola, Grippotyphosa, Pomona, Australis, Bataviae, Ballum, Cynopteri, Javanica, Panama, Pyrogenes, Sejroe, Tarassovi, Autumnalis, and Hebdomadis. The results demonstrated the antigenic relationship between CLEP 00060 and the Pomona serogroup (data not shown).

To identify the strain at the genomospecies level, we used different methodologies previously described as alternative tools to overcome the complex relatedness among serogroups, serovars, and species of *Leptospira*[9-11].

Multilocus sequence typing (MLST)[11] was performed and purified amplicons were sequenced using the Platform of deoxyribonucleic acid (DNA) Sequencing - RPT01A-PDTIS/ FIOCRUZ (http://plataformas.cdts.fiocruz.br/). Sequences were aligned with Clustal W and analyzed at the MLST website (http://pubmlst.org/leptospira). The analysis revealed that CLEP 00060 could be clustered with 100% similarity in the ST 117 and most likely belongs to Pomona serogroup (data not shown).

Pulsed Field Gel Electrophoresis (PFGE)[12] was performed using the restriction enzyme *Not*I (Sinapse Biotecnologia, Brazil). Fingerprints of the clinical isolate and different reference strains from serogroup Pomona were analyzed using GelCompar (Applied Maths) software, and the patterns produced were compared with the Dice coefficient and clustered using the unweighted pair-group method using arithmetic averages method. The dendrogram revealed a similarity index of approximately 92% between CLEP 00060 and strain 5621 (*L. kirschneri* serogroup Pomona serovar Mozdok) (**Figure 1**), indicating that these two strains belong to closely related serovars, according to the previously established criteria for PFGE interpretation[13].

Collection of Leptospira 00060 and two reference strains (*L. kirschneri* serogroup Grippotyphosa serovar Grippotyphosa strain Moskva V and *L. interrogans* serogroup Pomona serovar Pomona strain Pomona) were submitted to DNA extraction, followed by *fla*B amplification by polymerase chain reaction (PCR). The amplicons were digested separately by the restriction enzymes *Hind*III and *Hae*III (Sinapse Biotecnologia, Brazil) and the fingerprints were analyzed[9]. DNA was also used to amplify the *rrs* gene, and the amplified products were sequenced[10,14]. Sequences were compared with reference *Leptospira* sequences in the genome database of the National Center for Biotechnology Information (NCBI), using the basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The fingerprints generated by *fla*B-restriction fragment length polymorphism (RFLP) clearly showed the genetic relationship between the clinical isolate and *L. kirschneri* (**Figure 2**). Phylogenetic studies based on 16S rRNA gene sequences confirmed the result (data not shown).

Taken together, we assume that the isolate CLEP 00060 belongs to *L. kirschneri* serogroup Pomona and is closely related to serovar Mozdok, although more refined molecular methods, such as *sec*Y sequencing, and a comparison with a larger number of reference strains at PFGE could improve the identification. Even though patient sera presented low titers against serogroup Pomona in MAT, the identification of the strain can still be inferred because MAT presents low accuracy (about 33%) considering the correct prediction of the infective serogroup/serovar[15].

Based on the available information, serovar Mozdok is rarely isolated from humans in Brazil[7]. This is only the second report concerning the isolation of *L. kirschneri* putative serovar

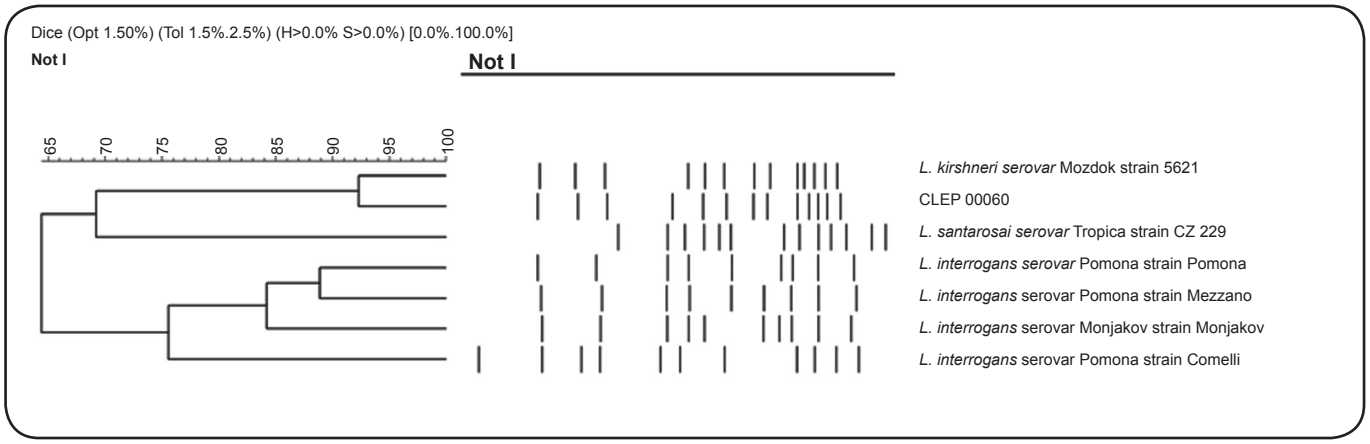

FIGURE 1 - Dendrogram generated by PFGE analysis showing the relationship among the clinical isolate CLEP 00060 and some reference strains from the serogroup Pomona. *L.:* *Leptospira;* PFGE: Pulsed Field Gel Electrophoresis**; CLEP:** Collection of Leptospira.

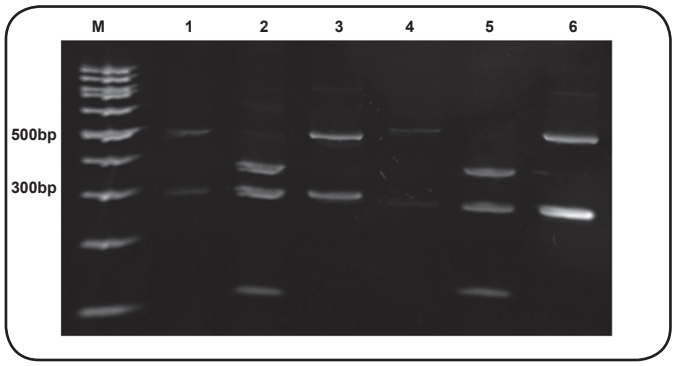

FIGURE 2 - Polyacrylamide gel electrophoresis (5%) of the PCR products resulting from the digestion of *fla*B by the restriction endonucleases *Hind*III and *Hae*III. M: 100bp DNA ladder (Sinapse Biotecnologia, Brazil); **1:** *L. kirschneri* reference strain Moskva V; **2:** *L. interrogans* reference strain Pomona; **3:** strain CLEP 00060 (1-3 digested with *Hae*III); **4:** *L. kirschneri* reference strain Moskva V; **5:** *L. interrogans* reference strain Pomona; **6:** strain CLEP 00060 (4-6 digested with *Hind*III). **PCR**: polymerase chain reaction; **DNA:** deoxyribonucleic acid; ***L.:*** *Leptospira;* **CLEP:** Collection of Leptospira.

Mozdok from a human case in a rural area of Brazil. The first report[7] describes the characterization of two isolates, from human and canine cases, in Pelotas, also in Rio Grande do Sul and about 240km from Santa Vitória do Palmar. Besides, the isolation of strains belonging to serogroup Pomona is more frequent in animals than in humans. Our finding reinforces the need for further studies with larger numbers of strains to define the epidemiological situation of leptospirosis in the rural areas of Brazil. It should be noted that awareness about the presence of uncommon serogroups and serovars in our territory would allow, according to the One Health approach, the prevention and control of leptospirosis by employing specific measures in accordance with our socio-economic scenario, bringing a long-term benefit to the Brazilian population.

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Conflicts of interest

The authors declare that have no conflicts of interest.

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