Short Report: Preliminary Characterization of *Mus musculus*–Derived Pathogenic Strains of *Leptospira borgpetersenii* Serogroup Ballum in a Hamster Model

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Abstract. Human and animal leptospirosis caused by *Leptospira* spp. belonging to serogroup Ballum has increased worldwide in the past decade. We report the isolation and serologic and molecular characterization of four *L. borgpetersenii* serogroup Ballum isolates obtained from *Mus musculus*, and preliminary virulence studies. These isolates are useful for diagnosis of leptospirosis and for epidemiologic studies of its virulence and pathogenic mechanisms.

Leptospirosis is the most widespread zoonosis in the world.¹ Over the past decade, leptospirosis has been recognized as an important neglected infectious disease.² In Latin America, Africa, and Asia the prevalence of leptospirosis is higher than in other continent mainly because of environmental conditions and distinct leptospiral reservoir species.³ Pelotas is a subtropical coastal city in southern Brazil and it has an annual incidence of > 50 cases of leptospirosis per 100,000 inhabitants, placing it among the cities with the highest incidence of leptospirosis in the country.⁴

Urban leptospirosis is caused mainly by *Leptospira interrogans* serogroup Icterohaemorrhagiae and *L. interrogans* serogroup Canicola. Interestingly, the increase in human leptospirosis associated with *L. borgpetersenii* serogroup Ballum has been reported worldwide. In a study conducted in the Caribbean archipelago of Guadeloupe, results emphasized a dramatic increase in the Ballum serogroup, which became the second most common infecting serogroup after Icterohaemorrhagiae.⁵ The same phenomenon has been observed in other countries. This serogroup has become the second most frequent cause of human leptospirosis in New Zealand⁶ and the third most frequent cause in Portugal.⁷ Furthermore, this serogroup appears to be newly established in Australia.⁸ In Cuba, serogroup Ballum has recently been reported as the main cause of human leptospirosis, leading to its use in two experimental, whole-cell, monovalent vaccines for human use.⁹

In nature, animal species such as mice and other rodents can act as reservoirs of *Leptospira* Ballum.¹⁰ Laboratory animals, especially mice and rats, may be experimentally infected with members of serogroup Ballum. However, they usually do not show clinical disease and leptospires may be excreted intermittently. In hamsters, members of the serogroup Ballum produce hemolytic disease caused mainly by hemolysin activity related to sphingomyelinases.¹¹

We report the isolation of four *L. borgpetersenii* serogroup Ballum strains from *Mus musculus*. We also report serologic, molecular, and preliminary virulence characteristics of these isolates.

Six mice were caught alive in traps left overnight near dwellings in a suburban area of Pelotas, Brazil. The animals

were immediately taken to the laboratory where they were humanely killed. Tissue samples were removed aseptically. Excised kidney material was macerated and suspended in liquid Ellinghausen-McCullough-Johnson-Harris medium (without antibiotics) for isolation. All cultures were incubated at 30°C and checked weekly for growth. Four animals had positive cultures (isolates were named 1E, 2E, 3E, and 4E), which were sub-cultured into liquid Ellinghausen-McCullough-Johnson-Harris medium for serologic and molecular typing.

Serogrouping was performed by using a panel of rabbit antisera at the Gonçalo Moniz Research Center (Fiocruz, BA, Brazil) as reported.¹² All isolates were classified as belonging to the Ballum serogroup. Species identification was accomplished by sequencing most of the 16S ribosomal RNA gene, as described.¹³ All isolates were classified as *L. borgpetersenii*. No molecular or serologic characterization at the serovar level was performed.

To determine if the isolates would produce infection in hamster model, groups of four 28-day-old animals were inoculated intraperitoneally with approximately 10⁸ leptospires of each isolate in a final volume of 1 mL. Animals were monitored daily for appearance of clinical signs. When moribund, they were humanely killed and subjected to necropsy. Kidneys were aseptically removed; one was macerated and suspended in liquid medium for re-isolation and the other was fixed in formalin buffer for histopathologic analysis. Animal procedures carried out in this study were reviewed and approved by the Committee for Animal Care and Use of Universidade Federal de Pelotas.

All isolates caused clinical signs, including evidence of dehydration, ruffled hair coat, decreased activity and isolation, within seven days of inoculation, and death shortly afterwards. Necropsy showed few pathologic findings in animals inoculated with isolates 1E, 2E, and 3E. However, isolate 4E produced severe lesions, with petechial hemorrhages in the lungs (Figure 1A), and jaundice. Staining with hematoxylin and eosin showed alveolar hemorrhage (Figure 1B) and nephritis. Staining with silver showed leptospires in the kidney (Figure 1C).

Samples from four of six captured animals produced positive cultures, indicating a high prevalence and risk of transmitting the disease to humans and other animals. All four isolates were obtained from the same area, host species, and period, but one of them, isolate 4E, exhibited a more aggressive pattern within the animal model of infection. Similar findings were observed for *L. borgpetersenii* isolates L550 and JB197, which belong to serovar Hardjo and have distinct phenotypes and virulence.¹⁴ Comparative genomic analysis of their genome sequences

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FIGURE 1. Histopathologic findings of leptospirosis in hamster tissue after intraperitoneal inoculation with 10⁸ strain 4E leptospires. **A**, Petequeal hemorrhages in the lungs (**arrows**) of hamsters seven days after infection. **B**, Prominent focal pulmonary hemorrhage in the lung (**arrows**) (hematoxylin and eosin stained). **C**, Hamster kidney tissue seven days after infection showing renal tubules with presence of *Leptospira* (**arrows**) (silver stained).

showed frameshift and point mutations that might be associated with different capacities to infect hamsters.¹⁵ This finding suggests that the *L. borgpetersenii* serogroup Ballum isolates describes in this study may represent distinct clonal subtypes. Further genetic analyses and serotyping will be required to fully characterize our isolates.

The recent increase in the number of cases of leptospirosis caused by leptospires of serogroup Ballum may be related to vaccination programs. Most veterinary vaccines do not include serogroup Ballum in their preparation. Vaccination may result in suppression of serogroups present in the vaccine, permitting less common species and serogroups to emerge. The same phenomenon occurs in humans, as was the case in Cuba.⁹

We recently reported isolation and characterization of seven leptospiral strains belonging to *L. interrogans* and *L. noguchii* from human and animal species from southern Brazil.^{12,16} The isolates obtained in this study will be used in similar experiments, enabling these isolates to be used in future studies of immunoprotection in the hamster model.

In summary, we have characterized four new isolates of *L. borgpetersenii* serogroup Ballum with various degrees of virulence. One of the isolates was highly virulent in the hamster model and caused pulmonary hemorrhage. Further characterization of these isolates is underway and may help to understand virulence and pathogenic mechanisms of *Leptospira* spp.

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