Leptospira kirschneri is one of the pathogenic species of the Leptospira genus. Human and animal infection from L. kirschneri gained further attention over the last few decades. Here we present the isolation and characterisation of Brazilian L. kirschneri serogroup Pomona serovar Mozdok strain M36/05 and the comparative genomic analysis with Brazilian human strain 61H. The M36/05 strain caused pulmonary hemorrhagic lesions in the hamster model, showing high virulence. The studied genomes presented high symmetrical identity and the in silico multilocus sequence typing analysis resulted in a new allelic profile (ST101) that so far has only been associated with the Brazilian L. kirschneri serogroup Pomona serovar Mozdok strains. Considering the environmental conditions and high genomic similarity observed between strains, we suggest the existence of a Brazilian L. kirschneri serogroup Pomona serovar Mozdok lineage that could represent a high public health risk; further studies are necessary to confirm the lineage significance and distribution.

Key words: L. kirschneri - serovar Mozdok - genomics

Leptospirosis is an emerging worldwide zoonosis which is caused by spirochetes of the Leptospira genus (Levett 2001). To date, the genus comprises 11 pathogenic species (Bourhy et al. 2014) of which L. interrogans is the most associated with human infection. Nevertheless, L. kirschneri infection has gained further attention over the last few decades.

Masuzawa et al. (2006) reported one of the first cases of L. kirschneri human infection by direct contact with southern flying squirrels imported from the United States; two Japanese workers were infected by L. kirschneri serovar Grippotyphosa from the imported American exotic pets. The L. kirschneri serogroup Pomona serovar Mozdok has also been related to human infection in Cuba (Obregón et al. 2007). In Europe, the serovar Mozdok is described as endemic in wild rodents (Majetic et al. 2014) and has also been associated with canine leptospirosis (Renaud et al. 2013).

In Brazil, L. kirschneri serogroup Pomona serovar Mozdok has recently been described as the causative agent of human and canine infection in different time points (Cunha et al. 2016). Here we present the isolation and characterisation of L. kirschneri serogroup Pomona serovar Mozdok strain M36/05 and the comparative genomic analysis with previously described Brazilian L. kirschneri serogroup Pomona serovar Mozdok human strain 61H.

The M36/05 strain was isolated from the kidney of a captured urban black rat (Rattus rattus) in Suzano, metropolitan region of São Paulo State, Brazil, in 2005. For isolation, 5 g of kidney sample was collected and homogenised in 50 mL of Sorensen saline, and 100 µL aliquots of 10^-1 to 10^-5 dilutions were inoculated into duplicate tubes containing EMJH (DIFCO, USA) enriched with 15% rabbit serum, 5-fluorouracil and nalidixic acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid. Once isolated, the strain was stored in Fletcher’s acid.
Genomic DNA was extracted and purified with illustra™ bacteria genomicPrep Mini Spin Kit (GE Healthcare do Brasil Ltda, São Paulo, Brazil) and used for paired-end library preparation with Nextera™ DNA Sample Prep Kit (Illumina®) and sequencing through Illumina® Miseq platform. The de novo assembly was performed with Geneious 8.1.8 (Biomatters Ltd, Auckland, New Zealand) and CLC Main Workbench 7.5.1 (CLC Bio, Denmark) and resulted in 44 scaffolds with a N50 of 241,247 bp.

The M36/05 draft genome (LLJK00000000) comprises ~4.46 Mb with overall GC content of 35.9%. Automatic genome annotation was performed with NCBI Prokaryotic Genome Annotation Pipeline. The basic annotation features identified in M36/05 strain are summarised in Table. With regard to the virulence genes, M36/05 presents genes encoding the main Leptospira virulence factors as lipoproteins and immunoglobulin-like proteins (ligA, ligB, ligC, lolC/D, lipA, lipL32 and loa22) and also flagellar proteins (flaA and flab subunits, fltG/F). In addition, antimicrobial resistance genes were also identified (tetA, ermA), including efflux pumps (norM, mdtA and qacA).

The M36/05 genome was compared to the Brazilian L. kirschneri serogroup Pomona serovar Mozdok human strain 61H (JSVJ00000000) through Mauve multiple genome aligner (Darling et al. 2004) and BLAST Ring Image Generator (BRIG) (Alikhan et al. 2011) and presented high symmetrical identity (98.86%) (Fig. 2). Due to the unavailability of a complete L. kirschneri reference genome, the chromosomes of the studied genomes were not individualised. The genetic content of strains is highly similar (Fig. 2A) and the few structural differences observed (Fig. 2B) could be due to differences in the applied assembly and ordering methodologies between the draft genomes. The absence of a reference genome for L. kirschneri still poses a challenge for assertive assembly and comparative analysis.

The in silico multilocus sequence typing (MLST) analysis was performed for the three Leptospira MLST protocols available (Table). Both strains presented similar results with ST98 and ST117 for Ahmed et al. (2006) and Boonsilp et al. (2013) protocols, respectively; these sequence types had already been associated with L. kirschneri serogroup Pomona serovar Mozdok. For the Varni et al. (2014) protocol, however, both strains presented a new allelic profile (7, 5, 22, 8, 7, 5) which originated a new ST101 that so far has only been associated with the Brazilian L. kirschneri serogroup Pomona serovar Mozdok strains. Considering the geographical and chronological distance between strains, since the 61H strain was isolated from human blood sample from Pelotas, in the metropolitan region of Rio Grande do Sul State, Brazil (~1,380 Km from São Paulo), in 2013 (Cunha et al. 2016), it is possible to infer that L. kirschneri serogroup Pomona serovar Mozdok has already been circulating in the southeast and southern regions of Brazil during the last two decades. It has apparently adapted to rodents as a reservoir and presents high virulent potential to humans. L. kirschneri serogroup Pomona serovar Mozdok has also been isolated from an asymptomatic dog in Pelotas (Cunha et al. 2016), suggesting that the serovar has already adapted to different reservoir hosts in urban areas.

In view of the environmental conditions and the high genomic similarity observed between strains, this may suggest that they could be a Brazilian L. kirschneri serogroup Pomona serovar Mozdok lineage that could represent a high public health risk; further studies are neces-

| Strain | Assembly statistics | Basic annotation features | Leptospira MLST schemes |
|--------|---------------------|--------------------------|------------------------|
|        | Scaffolds | N50 | Length | CG% | CDS | rRNAs | tRNAs | Ahmed et al. (2006) | Boonsilp et al. (2013) | Varni et al. (2014) |
| M36/05 | 44       | 241,247 | 4.46 Mb | 35.9 | 3,606 | 6 | 37 | ST98 | ST117 | ST101 |
| 61H    | 174      | 45,311 | 4.48 Mb | 35.9 | 3,629 | 5 | 38 | ST98 | ST117 | ST101 |
Fig. 2: whole-genome sequencing analysis of Brazilian *Leptospira kirschneri* serogroup Pomona serovar Mozdok strains. (A) BRIG plot displaying genomic similarity; (B) mauve alignment blocks.

Sary to confirm the lineage significance and distribution. Therefore, it should be included in the *Leptospira* battery of tests of national reference laboratories to enable proper identification and further epidemiological studies.

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