Comparative Immunology, Microbiology and Infectious Diseases 64 (2019) 7–9

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Genomic characterization and comparative analysis of *Leptospira kirschneri* serogroup Grippotyphosa UC5/2011, a strain isolated after mare abortion: Implications for genital animal leptospirosis

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ARTICLE INFO

Keywords:
Leptospira kirschneri
Next generation sequencing
Genital leptospirosis
Livestock

ABSTRACT

The genome of a Brazilian strain of *Leptospira kirschneri* serogroup Grippotyphosa isolated from a mare post-abortion was sequenced and analyzed. High symmetrical identity and few structural differences were found when compared with a European strain of the same serogroup, *L. kirschneri* serovar Valbuzzi strain 200702274. Genes associated with virulence and antimicrobial resistance were found. Knowledge of the virulence evolution of *Leptospira* remains limited, especially in diseases of the reproductive sphere. We highlight the importance of virulence studies in the sphere of genital leptospirosis.

1. Introduction

Leptospira in the genital tract has been described since the 1980s [1]. Several strains of *Leptospira* have been isolated from vaginal fluid, indicating the existence of a clinical disease related to reproduction in livestock [2]. Herein, we report the draft genome sequence of *L. kirschneri* serogroup Grippotyphosa strain UC5/2011 (UCR5RB1), which was isolated from urine of a mare post-abortion in Rio de Janeiro, Brazil [3].

2. Material and methods

Genomic DNA was obtained from pure culture in EMJH medium (Difco, USA) using the illustra™ bacteria genomicPrep Mini Spin Kit (GE Healthcare). A 300bp paired-end library was prepared using a Nextera™ DNA Sample Prep Kit (Illumina), and whole-genome DNA was sequenced through the Illumina MiSeq platform. A *de novo* assembly was generated using CLC Main Workbench 7.5.1 (CLC Bio, Qiagen) and GeneiousR10 (Biomatters Ltd), while the assembly metrics were accessed using QUAST [4]. Genome annotation was conducted with the NCBI Prokaryote Genome Annotation Pipeline (PGAP) [5]. The three MLST schemes were performed using the MLST v.1.8 server (www.cbs.dtu.dk/services/MLST) [6].

The genome of *L. kirschneri* serovar Valbuzzi strain 200702274 (NZ_AHOC00000000), isolated from a human sample that originated in France, was used to order the obtained scaffolds and for further comparative analysis using Mauve multiple genome aligner [7] and BLAST Ring Image Generator (BRIG) [8].

3. Results

The final assembly of the *L. kirschneri* strain UC5/2011 (UCR5RB1) draft genome (MVIS00000000) consisted of 89 contigs, comprising ˜4.3 Mb, with an overall G + C content of 35.9% and average coverage of 60.0 × . The results from the *de novo* assembly and an overview of the NCBI PGAP pipeline annotation are presented in Table 1. In the MLST analysis, the Ahmed et al. [9] and Boonsilp et al. [10] protocols yielded ST94 and ST110, respectively. In the Varni et al. [11] protocol, however, the strain UC5/2011 presented a new allelic profile (28, 25, 21, 8, 7, 7, 11) with a new ST100 that so far is exclusive to the Brazilian *L. kirschneri* serogroup Grippotyphosa equine strain.

Interestingly, the UC5/2011 strain showed high symmetrical

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https://doi.org/10.1016/j.cimid.2019.01.019
Received 10 August 2018; Received in revised form 21 January 2019; Accepted 23 January 2019
0147-9571/

Table 1
Genome metrics and overview of identified features in the genome of *L. kirschneri* serogroup Grippotyphosa strain UC5/2011 (UCR5RB1) (sequences length, N50 and L50 in bp).

Metrics	**Value**
Nº contigs	89
Length of the largest contig	276,671
Total length	4,336,701
N50	95,436
L50	16
G + C (%)	35.9
Features	**Occurrences**
CDSs	3,867
tRNAs	37
rRNAs	3
ncRNAs	2
CRISPR arrays	1
Pseudogenes	300

identity (> 98%) to the reference strain 200702274, and few structural differences were observed (Fig. 1), as expected. Due to the unavailability of a complete *L. kirschneri* reference genome, the chromosomes of the studied genomes were not individualized.

Genes related to antimicrobial resistance, such as erythromycin resistance (*erm*A) and tetracycline resistance (*tet*A), as well as genes coding for efflux pumps, putative multidrug efflux (*nor*M), multidrug resistance (*mdt*A), and antiseptic resistance (*qac*A) were identified. Moreover, genes encoding flagellar proteins and involved in virulence were also identified, such as *lig*A, *lol*C, *lol*D, *lip*A, *fli*G, and *fli*F.

4. Discussion

The scheme#3 ST94 and scheme#1 ST110 had already been associated with *L. kirschneri* strains, including the serovar Valbuzzi strain 200702274. Strains of scheme#1 ST110 has already been described in both domestic and wild animals, including in cattle from Belgium [12], synantropic small mammals from Malaysia [13], and in a hedgehog

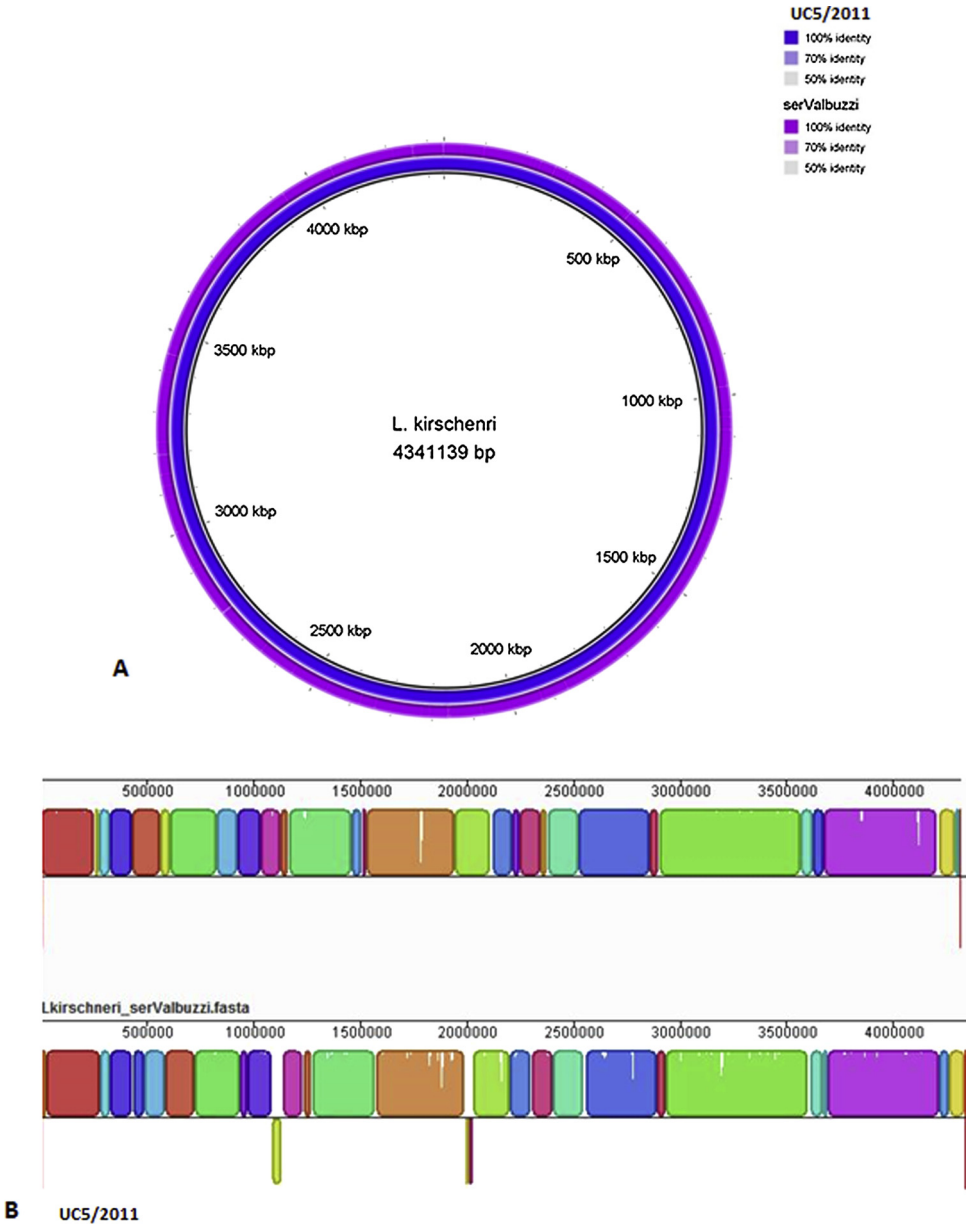

Fig. 1. Whole-genome sequencing analysis of Brazilian *L. kirschneri* serogroup Grippotyphosa strain UC5/2011 (UCR5RB1) and *L. kirschneri* sorovar Valbuzzi strain 200702274. A - BRIG plot displaying genomic similarity. B - Mauve alignment blocks.

case from Israel (strain Kipod 179) [10]. Interestingly, a case of bovine aborted foetuses was described in cattle from Belgium, being related to scheme#1 ST110 [12], suggesting a possible association between that strain of *L. kirschneri* and reproductive leptospirosis. Additionally, human cases were described in France (reference strain 200702274) and Holland (strain Duyster) [10]. Here, we highlight the zoonotic nature of scheme#1 ST110 strains and its wide circulation throughout the world.

UC5/2011 strain showed high symmetrical identity to the reference strain 200702274. Nevertheless, the high similarity is intriguing since the strain originated in an animal of European origin. By contrast, serogroup Grippotyphosa is not prevalent in tropical regions but is commonly found in Europe [3]. The strain studied was isolated from the urine of a mare after an abortion (seventh month of pregnancy). After necropsy, the foetus presents congenital jaundice and widespread petechial haemorrhages and leptospiral DNA was confirmed in fetal tissues [3]. Despite the European origin, the mare living in Brazil for the last two years (age eight years) and the follow-up of the other animals of the herd revealed 25% positivity in the pathogenic *Leptospira*-PCR [3]. Thus, it was inferred it was more likely to be a locally acquired infection. The source of infection cannot be detected. One possibility is the presence of synanthropic rodents circulating between animal stalls, since wild and synanthropic small mammals have already been described as infected with scheme#1 ST110 [10,13]).

Several genes related to antimicrobial resistance were found in UC5/2011 strain. The same resistance-related genes were found in a strain of *L. kirschneri* serogroup Pomona isolated from an urban rat in Brazil [14]. Although few studies have demonstrated the presence of antimicrobial resistance genes in *Leptospira*, the investigation of these genes is essential for understanding the spread and fixation of resistance alleles in pathogenic *Leptospira* species.

Initial findings also revealed the presence of genes encoding flagellar proteins and involved in virulence, such as *lig*A, *lol*C, *lol*D, *lip*A, *fli*G, and *fli*F. Some of these genes, such as *lig*A, are not found in saprophytic leptospires [15]; others, including *fli*G/F, are known to act on the motility of spirochetes [16]. Motility is reported as an important virulence determinant in *Leptospira* [17] which favoring entry and dissemination of pathogenic *Leptospira* in the host. The experimental inactivation of *fli*G and *fli*F genes prevent swimming and/or generate nonmotile bacteria [18,19], not allowing its internalization. The presence of these genes could be associated with reproductive disease observed in this herd since the other mares also presented a history of reproductive problems. In addition, the fact that the UC5/2011 strain has possibly caused abortion can be explained by the uterine immunesuppression and the complex immunoregulatory mechanisms in which pregnancy is involved. This contributes to the sparse knowledge of the virulence evolution of *Leptospira* in the sphere of reproductive disease, especially in animals of economic interest.

5. Conclusion

The present study presents the genome of a strain of *Leptospira kirschneri*, uncommon in horses, isolated from a mare after abortion. The results allow us to verify the circulation of scheme#1 ST110 strains among different countries and hosts, and add data for a better understanding of the epidemiology of animal leptospirosis. In addition, our study brings new insight for the control and treatment of genital leptospirosis.

Nucleotide sequence accession numbers - This whole genome shotgun project was deposited at DDBJ/EMBL/GenBank under the accessions MVIS00000000. The version described in this manuscript is MVIS00000000.1.

Funding

This work was supported by CAPES, National Council for Scientific and Technological Development (CNPq), São Paulo Research Foundation (FAPESP) [grant number AMM 2011/18290-0, LZM 2013/17136-2], FAPERJ [grant number LHJ E-26/202.766/2016].

Conflict of interest

The authors declare that there are no conflicts of interest.

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