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First Genome Sequence of *Leptospira interrogans* Serovar Pomona, Isolated from a Bovine Abortion

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Leptospirosis is a widespread zoonosis and a re-emergent disease of global distribution with major relevance in veterinary production. Here, we report the whole-genome sequence of *Leptospira interrogans* serovar Pomona strain AKRFB, isolated from a bovine abortion during a leptospirosis outbreak in Argentina.

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Leptospirosis is caused by spirochetes of the genus *Leptospira* (1). Serological classification indicates the presence of over 200 pathogenic serovars (2). The manifestations in livestock are mainly reproductive problems such as infertility and abortion (3). Cattle are usually maintenance hosts of serovar Hardjo throughout the world (4, 5), but the presence of other serovars was also demonstrated (6, 7). Remarkably, in Argentina the most frequent serovar in bovines is Pomona (8–11), while in other countries it is mostly associated with swine (12). However, there is scarce information about host-pathogen interactions with this serovar during livestock leptospirosis outbreaks. Thus, the availability of new genomic *Leptospira* sequences obtained from bovine isolates provides additional data for better understanding of this pathogen.

This work reports the draft genome sequence of *Leptospira interrogans* serovar Pomona strain AKRFB. The strain was isolated from a fetal bovine kidney in 2007, during a leptospirosis outbreak that affected a dairy herd in Buenos Aires, Argentina. The strain was characterized by serological and molecular methods. It belonged to serogroup Pomona and its associated genotype in Argentina (ST52) (10, 11). When evaluated in an animal model, the strain presented high virulence and caused neurological symptoms (13). Therefore, the strain was further evaluated as a candidate vaccine, satisfactorily protecting animals against challenge with the commercial vaccine and the other 3 field isolates (14). Upon incorporation of AKRFB to new commercial vaccines, there have been no records of isolates belonging to serogroup Pomona in vaccinated herds.

Genomic DNA was isolated using a standard chloroform isoamyl-alcohol extraction. Paired-end Nextera XT libraries were constructed and sequenced in an Illumina MiSeq sequencer. The quality trimming (15) applied to raw reads yielded 2,664,724 paired sequences. *De novo* assembly was done using SPAdes v3.6.2 (16) and reported 102 contigs >500 bp, the largest being 352,992 bp, with an N_{50} of 91,263 bp. Scaffolds were oriented using ABACAS (17) with the genome of *Leptospira interrogans* serovar Lai strain 56601 (18) as a reference. The final assembly comprised ~4.63 Mbp, 3,763 predicted genes, 37 tRNA copies, and a G+C content of 34.56%. The chromosome II was assembled into a single contig by SPAdes. The genome was annotated using PROKKA (19). The annotated scaffolds were compared with the same reference using BLAST and ACT (20) to analyze structural and gene content differences.

An MLST analysis (http://pubmlst.org/leptospira/) associated AKRFB with ST52. This profile also showed correlation to serogroup Pomona in worldwide strains as described previously (10).

ISFinder (21) was used to predict insertion sequences in the AKRFB draft genome as IS1500B, ISLin2, IS1500A, and, IS1501. The genome presents previously described virulence gene candidates, such as *lip*L32, *lip*L41, *lig*A, and *lig*B (2).

To our knowledge this is the first report of a complete sequence belonging to serogroup Pomona from a bovine abortion strain and the first sequence from an Argentinian isolate.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number LUHH00000000. The version described in this paper is version LUHH01000000.

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REFERENCES

1. **Levett PN**. 2001. Leptospirosis. Clin Microbiol Rev **14**:296–326. http://dx.doi.org/10.1128/CMR.14.2.296-326.2001.

2. **Lehmann JS, Matthias MA, Vinetz JM, Fouts DE.** 2014. Leptospiral pathogenomics. Pathogens **3:**280–308. http://dx.doi.org/10.3390/pathogens3020280.

3. **Lilenbaum W, Martins G.** 2014. Leptospirosis in cattle: a challenging scenario for the understanding of the epidemiology. Transbound Emerg Dis **61:**63–68. http://dx.doi.org/10.1111/tbed.12233.

4. **Schoonman L, Swai ES.** 2010. Herd and animal level risk factors for bovine leptospirosis in Tanga región of Tanzania. Trop Anim Health Prod **42:**1565–1572. http://dx.doi.org/10.1007/s11250-010-9607-1.

5. **Ellis WA, O'Brien JJ, Cassells J.** 2000. Role of cattle in the maintenance of *Leptospira interrogans* serovar Hardjo infection in Northern Ireland. Vet Rec **108:**555–557.

6. **Salgado M, Otto B, Sandoval E, Reinhardt G, Boqvist S.** 2014. Cross sectional observational study to estimate herd level risk factors for *Leptospira* spp. serovars in small holder dairy cattle farms in southern Chile. BMC Vet Res **10:**126. http://dx.doi.org/10.1186/1746-6148-10-126.

7. **Silva FJ, Conceicão WLF, Fagliari JJ, Girio RJS, Dias RA, Borba MR, Mathias LA.** 2012. Prevalence and risk factors of bovine leptospirosis in the state of Maranhão, Brazil. Pesqui Vet Bras **32:**303–312.

8. **Asociación Argentina de Veterinarios de Laboratorios de Diagnostico (AAVLD).** 2002. Informe sobre leptospirosis en la República Argentina. Comisión Científica sobre leptospirosis. AAVLD. Serie Enfermedades Transmisibles. Fundación Mundo Sano, Buenos Aires.

9. **Vanasco NB, Schmeling MF, Lottersberger J, Costa F, Ko AI, Tarabla HD.** 2008. Clinical characteristics and risk factors of human leptospirosis in Argentina (1999–2005). Acta Trop **107:**255–258. http://dx.doi.org/10.1016/j.actatropica.2008.06.007.

10. **Caimi K, Varni V, Melendez Y, Koval A, Brihuega B, Ruybal P.** 2012. A combined approach of VNTR and MLST analysis: improving molecular typing of Argentinian isolates of *Leptospira interrogans*. Mem Inst Oswaldo Cruz **107:**644–651. http://dx.doi.org/10.1590/S0074-0276201200500011.

11. **Varni V, Ruybal P, Lauthier JJ, Tomasini N, Brihuega B, Koval A, Caimi K.** 2014. Reassessment of MLST schemes of *Leptospira* spp. typing worldwide. Infect Genet Evol **22:**216–222. http://dx.doi.org/10.1016/j.meegid.2013.08.002.

12. **Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Díaz MM, Lovett MA, Levett PN, Gilman RH, Willig MR, Gotuzzo E, Vinetz JM, Peru-United States Leptospirosis Consortium.** 2003. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis **3:**757–771. http://dx.doi.org/10.1016/S1473-3099(03)00830-2.

13. **Koval A, López S, Nardello M, Vena MM, Margueritte J.** 2007. Isolation, serotyping and evaluation of immunogenicity and cross-protection of a field strain of *Leptospira interrogans* belonging to the Pomona serogroup. Vet Arg **24:**333–340.

14. **López S, Koval A.** 2011. Evaluation of vaccine strain AKRFB of *Leptospira interrogans* serovar Pomona against 3 virulent field strains isolated from bovines in Argentine. Vet Arg **28:**1–6.

15. **Bolger AM, Lohse M, Usadel B.** 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30:**2114–2120. http://dx.doi.org/10.1093/bioinformatics/btu170.

16. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA.** 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol **19:**455–477. http://dx.doi.org/10.1089/cmb.2012.0021.

17. **Assefa S, Keane TM, Otto TD, Newbold C, Berriman M.** 2009. ABACAS: algorithm-based automatic contiguation of assembled sequences. Bioinformatics **25:**1968–1969. http://dx.doi.org/10.1093/bioinformatics/btp347.

18. **Ren SX, Fu G, Jiang XG, Zeng R, Miao YG, Xu H, Zhang YX, Xiong H, Lu G, Lu LF, Jiang HQ, Jia J, Tu YF, Jiang JX, Gu WY, Zhang YQ, Cai Z, Sheng HH, Yin HF, Zhang Y, Zhu GF, Wan M, Huang HL, Qian Z, Wang SY, Ma W, Yao ZJ, Shen Y, Qiang BQ, Xia QC, Guo XK, Danchin A, Saint Girons I, Somerville RL, Wen YM, Shi MH, Chen Z, Xu JG, Zhao GP.** 2003. Unique and physiological and pathogenic features of *Leptospira interrogans* revealed by whole genome sequencing. Nature **422:**888–893. http://dx.doi.org/10.1038/nature01597.

19. **Seemann T.** 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics **30:**2068–2069. http://dx.doi.org/10.1093/bioinformatics/btu153.

20. **Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J.** 2005. ACT: the Artemis comparison tool. Bioinformatics **21:**3422–3423. http://dx.doi.org/10.1093/bioinformatics/bti553.

21. **Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M.** 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res **34:**D32–D36. http://dx.doi.org/10.1093/nar/gkj014.