

TITLE: MICROEVOLUTIONARY HISTORY OF *LEPTOSPIRA INTERROGANS* BASED ON THEIR CRISPR-CAS INVENTORY

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ABSTRACT:

Leptospirosis is a bacterial zoonosis which impacts both human and animal health worldwide and is caused by pathogenic members of the genus *Leptospira*. Taxonomic classification of the genus includes 8 pathogenic species and more than 200 serovars. *L. interrogans* is an important pathogen and despite having many sequenced genomes, the evolutionary relation between the serovars and strains are still not fully understood. The CRISPR-Cas system is an adaptive and heritable immunity mechanism composed of Cas proteins and an array of repeats interspersed with spacers. The spacers represent chronological records of element-infecting cells, and the comparative analysis of CRISPR locus allows for the assessment of adaptive dynamics and the microevolution of bacterial populations. Here, we analyzed CRISPR-Cas systems from the genomes of *L. interrogans* serovar Hardjo str Norma, *L. interrogans* serovar Hardjo str Hardjo-prajitno, *L. interrogans* str Brem, *L. interrogans* serovar Lai, *L. interrogans* serovar Linhai and *L. interrogans* serovar Copenhageni. The CRISPR array were predicted using CRISPRfinder and the spacers were compared using a Perl script. All the spacers were searched for their similarity to GenBank using BLASTN. *L. interrogans* genomes were found to encode at least two CRISPR-Cas systems, type I-B and type I-C. The type I-C have the Cas coding genes *cas3*, *cas5*, *cas8c*, *cas7*, *cas4*, *cas1*, *cas2* and a small CRISPR array. Regarding CRISPR locus from type I-B, we observed an operon composed of *cas1*, *cas2*, *cas6*, *cas3*, *cas8a1*, *cas7*, *cas5* and a CRISPR array with a conserved repeat between serovars. The strains Norma, Brem and Hardjo-prajitno have a mobile element (IS110) flanking the CRISPR operon. Furthermore, the spacers from these strains are shared on the same order, different from the observed considering the spacers repertoire of serovars Lai, Linhai and Copenhageni. Together these finds suggest that CRISPR locus from Norma, Brem and Hardjo-prajitno may be acquired from horizontal gene transfer. It is important to mention that strains Norma and Hardjo-prajitno belong to the same serovar Hardjo and Brem has no serovar defined yet. Some of the spacers found in the *L. interrogans* genomes target regions from the two plasmids of serovar Linhai, including regions of phage protein and a prophage. This suggest that *L. interrogans* Norma, Brem, Hardjo-prajitno and Lai have the protective mechanism against invasion of Linhai plasmids and probable phages.

Keywords: *Leptospira interrogans*, CRISPR-Cas system, microevolution

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