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Genotypes of *Leptospira* spp. strains isolated from dogs in Buenos Aires, Argentina

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**KEYWORDS**
Molecular characterization; MLVA; *Leptospira* spp.

**Abstract**
Leptospirosis is an infectious disease of wide global distribution, which is endemic in Argentina. The objective of this study was to obtain the genetic profiles of *Leptospira* spp. strains isolated from clinical cases of dogs in the province of Buenos Aires by the multiple-locus variable-number tandem repeat analysis (MLVA). Eight isolated canine strains were genotyped by MLVA, obtaining the identical profile of *Leptospira interrogans* serovar Canicola Hond Utrecht IV in the strains named Dogy and Mayo. The strains named Bel, Sarmiento, La Plata 4581 and La Plata 5478 were identical to the profile of the genotype of *L. interrogans* serovar Portlandvere MY 1039. The strain named Avellaneda was identical to the genotype profile of *L. interrogans* serovar Icterohaemorrhagiae RGA and the strain named SB had the same profile as the *L. interrogans* serovar Pomona Baires genotype and was similar to the profile of serovar Pomona genotype. It would be useful to include a larger number of isolates from different dog populations in various provinces of Argentina and to characterize the genetic profiles of the strains circulating in the country. The information obtained will be useful for the control of leptospirosis in the dog population.

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Leptospirosis is a neglected zoonosis, which is endemic in most tropical and subtropical regions of the world, being most of the cases reported in Asia and the Americas. This disease is often misdiagnosed in humans suffering from other febrile diseases such as meningitis and dengue. Yearly, an estimate of 500,000 cases is diagnosed worldwide and the mortality rate is over 10%13. The causing bacteria of this zoonosis are pathogenic strains belonging to the order Spirochaetales, family Leptospiraceae and genus Leptospira spp. Knowledge of the circulating pathogenic strains that commonly cause disease within a particular geographic region is important for vaccine efficacy. For instance, in Europe a recent tetravalent Leptospira spp. vaccine for dogs was studied due to the fact that clinical evidence showed that a bivalent vaccine was not appropriate for the current epidemiological scenario. This vaccine is directed against Leptospiro interrogans serogroups Canicola, Icterohaemorrhagiae, Grippotyphosa and Australis7. Dogs are primary infected by L. interrogans and L. kirschneri. Since the introduction of the vaccine 30 years ago, the most relevant serogroups had been Canicola and Icterohaemorrhagiae. Currently more serogroups such as Grippotyphosa, Pomona, Bratislava and Autumnalis are affecting dogs. The infecting serovars may vary among canine populations depending on exposure to infected wild or domestic animal reservoir hosts5. In Argentina all vaccines for canine leptospirosis are against serovar Canicola and serovar Icterohaemorrhagiae and some also include serovar Castellonis and serovar Grippotyphosa6.

Clinical signs and severity of canine leptospirosis vary depending on the geographic population, the infecting serovar and the dogs'13 immune response. The following clinical signs can indicate a leptospirosis infection in dogs: renal or hepatic failure, uveitis, pulmonary hemorrhage, acute febrile illness or abortion2,5,6. In Argentina, serological studies using the microagglutination test (MAT) found seroreactivity against the following serovars in dogs: Bataviae, Canicola, Castellonis, Icterohaemorrhagiae, Grippotyphosa, Pyrogenes, Pomona and Tarassovi5. Serological studies in dogs from Buenos Aires have shown seroreactivity against serovars: Canicola2,3,5, Castellonis5, Icterohaemorrhagiae2,3,5, Grippotyphosa5, Tarassovi5, Pomona5 and Cynopteri14. Previous isolations of pathogenic Leptospira spp. in dogs in Argentina include L. interrogans belonging to serogroups Canicola, Icterohaemorrhagiae and Pyrogenes5.

The importance of following infection control guidelines was highlighted after a small animal veterinarian got infected with a virulent Leptospira sp. strain as he was examining a pet rat for fleas, not wearing gloves to protect his hands which had abrasions from gardening1,12. Similarly, a veterinarian could also become infected when examining a dog.

A total of 8 strains isolated from household dogs were used in this study. Two of them (La Plata 4581, and La Plata 5478) were isolated from dogs in the city of La Plata, Buenos Aires Province. The other six strains were isolated from dogs in the periurban area of Buenos Aires city (Table 1). The reference strains of L. interrogans used in this study were: serovar Pomona Pomona (serogroup Pomona), serovar Copenhageni M20 (serogroup Icterohaemorrhagiae), serovar Icterohaemorrhagiae RGA and Ictero I of the serogroup Icterohaemorrhagiae, serovar Canicola Hond Utrecht IV (serogroup Canicola) and serovar Portlandvere MY 1039 (serogroup Canicola).

The reference strains and isolated strains were grown in Fletcher media (Difco Laboratories) at 28°C. For the DNA templates used in the MLVA strain, typing procedures performed with the primers flanking of loci VNTR4, VNTR7, VNTR9, VNTR10, VNTR19, VNTR23 and VNTR31 were used to discriminate strains of L. interrogans6,9,10. To discriminate between reference strains RGA, M20 and Icterohaemorrhagiae we used primers to flank loci VNTR4, VNTR7, VNTR9, VNTR10, VNTRLb4 and VNTRLb511. The
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The final volume (50 μl) of each reaction mixture contained PCR Buffer (20 mM Tris-HCl pH 8.4; 50 mM KCl), 200 μM deoxinucleoside triphosphates, 2 μM each corresponding primer, 2 mM MgCl₂, 1.25 U Taq DNA polymerase (Invitrogen) and 5 μl DNA template. PCRs were carried out in a Thermo Scientific PxE 0.2 Thermal Cycler as follows: 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 55ºC for 30 s and extension at 72ºC for 90 s, with a final cycle at 72ºC during 10 min. Amplified samples (15 μl) were revealed by electrophoresis in a 2% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) with ethidium bromide at 100 V for 50 min. Amplified DNA bands were visualized upon UV light exposure (Uvi Tec transiluminator BTS-20.M). Amplicon sizes were estimated using CienMarker (Biodynamics) and the GelAnalyzer 2010a program. The following formula was used to calculate the repeat copy number: Number of repeat (bp) = [Fragment size (bp)-Flanking regions (bp)]/Repeat size (bp). Repeat copy numbers were rounded down to the closest whole numbers. If the copy number was less than one it was rounded to zero.

The genotypes obtained were used to assemble a phylogenetic tree with the Mega 5.2 software. The tree was constructed by the neighbor-joining method using seven marker loci for the *L. interrogans* strains (Fig. 1). Bootstrap consensus values are not indicated because no sequence analysis was used.

A total of eight isolated strains from clinical cases of dogs were genotyped in this study, all belonging to *L. interrogans*, two of which were identical to the MLVA profile of serovar Canicola Hond Utrecht IV (Dogy and Mayo), and four identical to the serovar Portlandvere MY 1039 MLVA pattern (La Plata 4581, La Plata 5478, Sarmiento and Bel), all of them belonging to serogroup Canicola. One strain (SB) was identical to the MLVA profile of serovar Pomona Baires and similar to *L. interrogans* serovar Pomona Pomona. The strain with the identical MLVA profile of *L. interrogans* serovar Icterohaemorrhagiae RGA (Avellanaeda) was discriminated using VNTR4, VNTR7, VNTR10, VNTRLb4 and VNTRLb5 loci, with copy numbers (2,1,7,-,-).

The results obtained in this study show diversity among *Leptospira* spp. strains isolated from clinical cases of dogs in Buenos Aires Province (Fig. 1). The genetic profiles show the diversity of the circulating pathogenic strains of *L. interrogans* in the Province of Buenos Aires. However, more isolations from different provinces and cities have to be made to confirm the actual scenario referring to circulating pathogenic strains in the country. Vaccination against canine leptospirosis is not compulsory in Argentina and different vaccines are available, most of which are imported from the US and Europe. We found two genotypes belonging to serogroup Canicola, *L. interrogans* serovar Canicola Hond Utrecht IV and *L. interrogans* serovar Portlandvere MY1039, one genotype belonging to serogroup Pomona identical to *L. interrogans* serovar Pomona Baires, and one genotype belonging to serogroup Icterohaemorrhagiae, *L. interrogans* serovar Icterohaemorrhagiae RGA. These findings highlight the importance of characterizing the circulating genotypes in dogs, since they share the same environment with humans, and therefore pose a risk of transmission, which in this zoonosis implies disseminating *Leptospira* spp. into the environment through urine.

**Ethical responsibilities**

**Protection of human and animal subjects.** The authors declare that no experiments were performed on humans or animals for this investigation.
Confidentiality of Data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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