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INFORME BREVE

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

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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 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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PALABRAS CLAVE
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Genotipos de cepas de *Leptospira* spp. aisladas de perros en Buenos Aires, Argentina

Resumen
La leptospirosis es una enfermedad infecciosa de amplia distribución global; endémica en Argentina. El objetivo de este estudio fue obtener los perfiles genéticos de las cepas de *Leptospira* spp. aisladas de casos clínicos de perros provenientes de la provincia de Buenos Aires, empleando el análisis de repeticiones en tándem de número variable en múltiples locus [*multiple-locus variable-number tandem repeats analysis* (MLVA)]. Fueron genotipificadas por MLVA ocho cepas aisladas de perros. Se obtuvo un perfil idéntico al de *Leptospira interrogans* serovar Canicola Hond Utrecht IV en las cepas denominadas Dogy y Mayo. Las cepas denominadas Bel, Sarmiento, La Plata 4581 y La Plata 5478 mostraron un perfil idéntico al genotipo de *L. interrogans* serovar Portlandvere MY 1039. La cepa denominada Avellaneda presentó un perfil idéntico al genotipo *L. interrogans* serovar Icterohaemorrhagiae RGA, y la cepa denominada SB mostró un perfil idéntico al genotipo de *L. interrogans* serovar Pomona Baires y similar al serovar Pomona Pomona. Sería de gran utilidad incluir un mayor número de cepas provenientes de distintas poblaciones caninas de diversas provincias de Argentina a fin de conocer los perfiles de las cepas circulantes en el país. La información así obtenida contribuirá al control de la leptospirosis en la población canina.

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%[15]. 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 *Leptospira interrogans* serogroups Canicola, Icterohaemorrhagiae, Grippotyphosa and Australis[7]. 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 hosts[7]. In Argentina all vaccines for canine leptospirosis are against serovar Canicola and serovar Icterohaemorrhagiae and some also include serovar Castellonis and serovar Grippotyphosa[6].

Clinical signs and severity of canine leptospirosis vary depending on the geographic population, the infecting serovar and the dogs[12] immune response. The following clinical signs can indicate a leptospiral infection in dogs: renal or hepatic failure, uveitis, pulmonary hemorrhage, acute febrile illness or abortion[2,5,6]. 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 Tarassovi[5]. Serological studies in dogs from Buenos Aires have shown seroreactivity against serovars: Canicola[2,3,5], Castellonis[5], Icterohaemorrhagiae[2,3,5], Grippotyphosa[5], Tarassovi[5], Pomona[5] and Cynopteri[14]. Previous isolations of pathogenic *Leptospira* spp. in dogs in Argentina include *L. interrogans* belonging to serogroups Canicola, Icterohaemorrhagiae and Pyrogenes[4].

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 gardening[1,12]. 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 No.1 (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 28ºC. 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. interrogans*[8,9,10]. To discriminate between reference strains RGA, M20 and Ictero I of the serogroup Icterohaemorrhagiae we used primers to flank loci VNTR4, VNTR7, VNTR10, VNTRLb4 and VNTRLb5[11]. The