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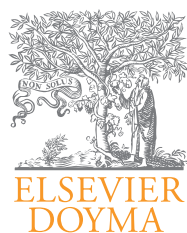
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INFORME BREVE

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

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KEYWORDS

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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

Caracterización
molecular;
MLVA;
Leptospira spp.

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.

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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%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 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 hosts⁷. 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 dogs¹² 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 Tarassovi⁵. Serological studies in dogs from Buenos Aires have shown seroreactivity against serovars: Canicola^{2,3,5}, Castellonis⁵, Icterohaemorrhagiae^{2,3,5}, Grippotyphosa⁵, Tarassovi⁵, Pomona⁵ and Cynopteri¹⁴. Previous isolations of pathogenic *Leptospira* spp. in dogs in Argentina include *L. interrogans* belonging to serogroups Canicola, Icterohaemorrhagiae and Pyrogenes⁴.

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 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. 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 VNTRLb5¹¹. The

Table 1 Localities and sources of pathogenic *Leptospira* spp. strains isolated from dogs used in this study

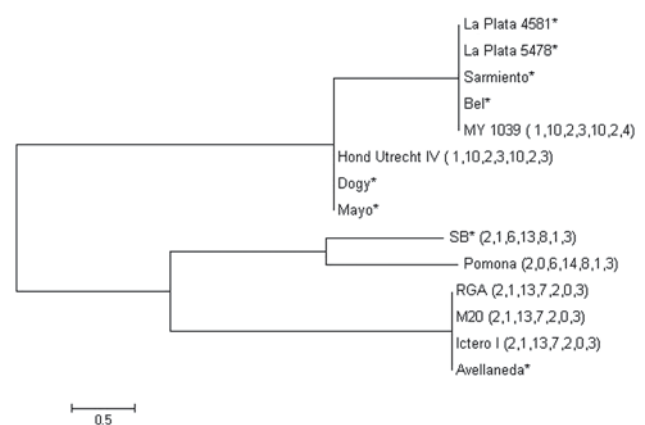
Strain	Source	Locality and (year)
Bel	Isolated from urine	Periurban area of Buenos Aires city, Province of Buenos Aires (2006)
Sarmiento	Isolated from urine	Periurban area of Buenos Aires city, Province of Buenos Aires (2007)
La Plata 4581	Isolated from blood	La Plata city, Buenos Aires Province (2012)
La Plata 5478	Isolated from urine	La Plata city, Buenos Aires Province(2012)
Avellaneda	Isolated from urine	Periurban area of Buenos Aires city, Province of Buenos Aires (2007)
Mayo	Isolated from urine	Periurban area of Buenos Aires city, Province of Buenos Aires (2008)
Dogy	Isolated from urine	Periurban area of Buenos Aires city, Province of Buenos Aires (2009)
SB	Isolated from fetus	Periurban area of Buenos Aires city, Province of Buenos Aires (2008)
Total 8		

final volume (50 µl) of each reaction mixture contained PCR Buffer (20 mM Tris-HCl pH 8.4; 50 mM KCl), 200 µM deoxynucleoside 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 Px2 Thermal Cycler as follows: 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 90 s, with a final cycle at 72°C 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 transilluminator 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 software¹³. 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 (Avellaneda) 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

**Figure 1** Unrooted Neighbor-Joining tree using 7 loci repeats of *Leptospira interrogans*. Isolated strains are marked with *.

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 Baires¹⁰ (2,1,6,13,8,1,3), 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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