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Research Article

Serological Conversion for Anti-Leptospira Antibodies among Domestic Dogs from Southern Chile, A Prospective Study

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Keywords

- Anti-*Leptospira* antibodies; Dogs; Prospective study; Rate of serological conversion

Abstract

Background: Infection by *Leptospira* is relevant in canine medicine. However, prospective studies about leptospirosis in dogs are scarce worldwide.

Methods: A prospective study among owned domestic dogs from southern Chile was performed by the Microscopic Agglutination Test: 1) to estimate the rate of serological conversion for anti-*Leptospira* antibodies in a 6 to 9 month follow-up period, 2) to determine the reactive serovars and, 3) to measure antibody titers in seropositive dogs. There were two samplings: in the first, 192 animals were sampled and 50 re-sampled dogs constituted the second.

Results: The rate of serological conversion in the follow-up period was 12.0% (95% Confidence Interval=2.9-21.0%). In the first sampling, the most reactive serovars were Ballum and Canicola. In the second sampling, the most reactive serovar was Icterohaemorragiae. In both samplings, the antibody titers ranged between 1:100 and 1:800, with predominance titers of 1:100 and 1:200.

Conclusions: The relatively high rate of serological conversion suggests that the exposure to *Leptospira* in dogs is present in southern Chile, with a possible endemic presentation of the seropositivity. Preventive measures such as vaccination and to reduce the exposition of pet dogs to reservoirs of the bacteria must be taken, as well to increase the awareness about *Leptospira* infection among public health institutions, veterinary practitioners and dog owners.

ABBREVIATIONS

MAT: Microscopic Agglutination Test; n: Sample Size; 95% CI: 95% Confident Interval

INTRODUCTION

Leptospirosis is an emerging neglected disease caused by pathogenic species of the genus *Leptospira* [1]. Leptospires are shed in urine of animals and infection is initiated after exposure to contaminated water, or by infiltration of the intact skin following the contact with contaminated urine [2].

Concerns about animal leptospirosis are related to risk situations in the human-animal-ecosystem interface, as well as the economic burden in veterinary and public health [1]. Domestic dogs can become reservoir hosts of the bacterium, being a link in the transmission of the disease to people [3,4]. Since clinical features of canine leptospirosis vary from subclinical infections to multiorgan involment with renal, hepatic, hematologic and pulmonary failure [5,6], specific diagnostic tests are required to confirm the disease status [7,8]. Therefore, monitoring the incidence of canine leptospirosis is a very difficult task [9]. If a diagnosis of leptospirosis occurs, veterinarians must inform to pet owners the zoonotic potential of the disease. To prevent the infection, annual vaccination of dogs is recommended, as well as to perform rodent control and, to reduce the access to potential sources of infection, such as standing water, muddy soil and humid areas [10].

In Chile, there are some published cross-sectional studies on leptospirosis in domestic dogs, in which prevalences of 14.8% [11], 21.3% [12], 25.2% [13] and 38.3% [14] were reported. However, no prospective epidemiologic studies that include data about leptospiral serological conversions have been performed in the country. Assessing if new seropositive animals appear at a certain period will provide important information to improve the knowledge on the dynamics of the occurrence of *Leptospira* infection in canines. This will also help, for example, to define measures to prevent the transmission of the bacterium. The aims of this study were: 1) to estimate the rate of serological conversion for anti-*Leptospira* antibodies in a group of dogs from southern Chile in a 6 to 9 month follow-up period, 2) to determine the reactive serovars and serogroups in seropositive dogs and, 3) to measure antibody titers with MAT (Microscopic Agglutination Test).

MATERIALS AND METHODS

Study areas

The study areas were the cities of Valdivia (39° 48´South and 73° 14´ West) and Paillaco (40° 02´ South and 72° 52´ West) [15] located in Los Ríos Region in southern Chile (Figure 1).

Sampling

In this study, blood samples of domestic dogs were taken in two sampling periods. The first was performed between January and November and the second was 6- 9 months later. Males and females dogs, older from 2 months of age and canines of different breeds were sampled. Dog owners were informed about the objectives of the research and voluntarily agreed to be part of the study.

In the first sampling, the sample size was determined considering an approximate population of 16.772 dogs in the cities of Valdivia and Paillaco altogether [16,17]. With an expected prevalence of 14.8% [11], an acceptable error of 5% and a confidence level of 95%, the estimated sample size were 192 animals.

The second sampling consisted in 50 dogs that had been present in the first sampling, which still lived in the cities of Valdivia and Paillaco and whose owners agreed to participate in the survey again. Loss to-follow-up occurred mostly because the owners refused to participate for a second time in the study (n=67). Death of the animals was also a cause of loss-to-follow-up (n=27). The unsuccessful attempt to contact the owners of the animals due to changes in their contacts or addresses also accounted to the reduction of the second sampling (n=48).

Sample collection

In both samplings, blood samples (1-2 ml) were randomly collected by venipuncture from dog patients in veterinary clinics and from canines enrolled during home visits. Healthy animals and dogs attending at veterinary clinics for different reasons were included in the study.

Ethical approval

The methods for animal handling and blood extraction obtained the certification of the bioethics committee at the Universidad Austral de Chile (certification #10-2012).

Serology

The presence of anti-*Leptospira* antibodies was detected using MAT. All samples of the first and the second samplings were tested against a panel of live cultures of reference *Leptospira* serovars used as antigens following standard recommendations [16]. Six serovars were included in the panel (Table 1) because they are among those that occur in animals in Chile according to the literature [11,18].

To broaden the panel of serovars analyzed, 26 of the MAT-negative samples of the first sampling were re-analyzed with 12 different serovars (Table 1) with the methodology described by Faine [19], World Health Organization and International Leptospirosis Society [20]. Is important to note that this large panel of serovars was not available for laboratory use at the time of the first sampling, therefore, only the samples in which the serum volume was sufficient to perform MAT a second time were re-analyzed.

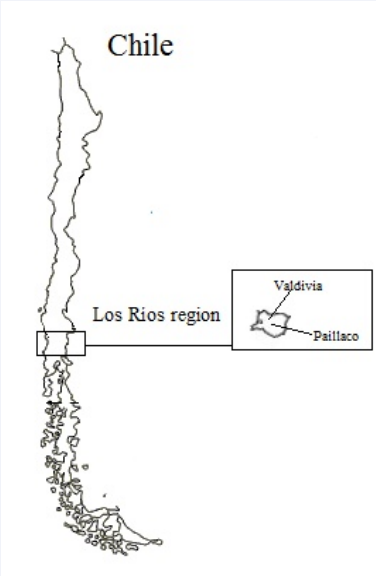

Figure 1 Map of the study area.

The conditions in MAT to categorize a sample as with anti-*Leptospira* antibodies (case definition) were the following [21]:

1. For unvaccinated dogs, a single titre of 1:100 or greater in the diagnostic test.
2. In the vaccinated dogs, the antibody titers and the time since vaccination were considered. For animals vaccinated from 1 to 3 months before the collection of the blood sample, titres of 1:400 or higher were considered as positives and for dogs vaccinated 3 to 12 months before sampling, titres of 1:200 or higher were considered as positives. The vaccine most commonly used in Chile contains *L. interrogans* serovars Canicola and Icterohaemorrhagiae. Other vaccines may contain *L. interrogans* serovar Pomona and *L. kirschneri* serovar Grippotyphosa.
3. For dogs reacting positively in MAT for more than one serovar, the serovar with the highest titre was specified as the cause of the serological reaction to *Leptospira* and reactions to different serovars at the same titre were considered coagglutinations.

EPIDEMIOLOGIC QUESTIONNAIRE

A questionnaire developed during this study allowed gathering information about the breed (purebred or mixed-breed), size breed (small, medium, large), gender, age (according the information by dog owners, clinical records or dental chronometry), vaccination status (vaccinated or non-vaccinated, according clinical records or the data specified by the veterinary practitioners), regular submission to veterinary care (yes/no) and presence/absence of clinical signs compatible

Table 1: Reference strains, serovars and serogroups of *Leptospira* species used in MAT.

Panel	Reference strain	Serovar	Serogroup	Specie
Panel 1	Hardjo pratijno	Hardjo	Sejroe	*L. interrogans*
	Pomona	Pomona	Pomona	
	Hond Utrech IV	Canicola	Canicola	
	Verdum	Icterohaemorragiae	Icterohaemorragiae	
	Akiyami A	Autumnalis	Autumnalis	
	S102	Ballum	Ballum	*L. borgpetersenii*
Panel 2	Salinem	Pyrogenes	Pyrogenes	*L. interrogans*
	3705	Wolfii	Sejroe	
	Swart	Bataviae	Batavie	
	Ballico	Australis	Australis	
	Hebdomadis	Hebdomadis	Hebdomadis	
	Perepelitsin	Tarassovi	Tarassovi	*L. borgpetersenii*
	Veldrat Batavia 46	Javanica	Javanica	
	M 84	Sejroe	Sejroe	
	Moskva V	Grippotyphosa	Grippotyphosa	*L. kirschneri*
	3522 C	Cynoptery	Cynoptery	
	CZ 214K	Panama	Panama	*L. noguchii*
	Patoc 1	Patoc	Semaranga	*L. biflexa*

Table 2: Individual characteristics, antibody titers, *Leptospira* serovars and serogroups found in dogs with serological conversion.

Dog No.	Breed	Size-breed	Gender*	Age (years)	Clinical signs	Antibody titers	Serovar*/ serogroup*
1	Fox terrier	Small	M	2	No	1:800	Ict/Ict
2	Mixed-breed	Small	M	3	No	1:100	Ict/Ict
3	Mixed-breed	Small	M	8	No	1:100	Bal/Ball
4	Mixed-breed	Small	M	1	No	1:100	Hard/Sej
5	Mixed-breed	Medium	M	8	No	1:100	Aut/Aut
6	Cocker spaniel	Medium	F	12	No	1:100	Pom/Pom

*M= male, F=female
*Serovars: Ict= Icteroaemorragiae, Ball= Ballum, Hard= Hardjo, Aut= Autumnalis.
*Serogroups: Ict= Icteroaemorragiae, Ball= Ballum, Sej= Sejroe, Aut= Autumnalis

with leptospirosis. Regarding this, animals were considered suspected of disease if one or more of the following clinical signs were present: fever, icterus or polyuria. Alterations in the biochemical profile (if this was available) reflecting renal and/or hepatic compromise, such as increased serum urea or creatinine, alanine aminotransferase, aspartate aminotransferase and/or alkaline phosphatase were considered as putative evidences of leptospirosis. The questionnaire and clinical examination referred to the two sampling periods, and both were applied by a veterinary practitioner.

Definition of dogs with serological conversion

In this study, a "serological conversion" is defined as the detection of anti-*Leptospira* antibodies through MAT in a sample taken from a dog that tested negative in the first sampling. Dogs were considered "serological reactors" for MAT if they met any of the criteria for positive samples previously indicated, or when they tested positive for a particular serovar for which they were negative in the first test.

Rate of serological conversion

The rate of serological conversion was calculated as the number of dogs with serological conversion in the follow-up period over the number of sampled animals in both occasions. The 95% Confidence Intervals (95% CI) were estimated by the method described by Pretie and Watson [22].

RESULTS AND DISCUSSION

In the present study, we denominate our estimate as "rate of serological conversion" considering the small sample size of the re-sampled animals (n=50). Nevertheless, the rate of serological conversion was 12.0% (95% CI= 2.9-21.0) in the follow-up period. These suggest that the exposure to the bacterium is present with time in southern Chile with a rate relatively high. Considering that pets could be good sentinels to identify zoonotic

pathogens for humans [23], the rate of serological conversion estimated in this study highlight the need of the implementation of a surveillance system for leptospirosis in domestic dogs in Chile and, also emphasize the need to increase the awareness about leptospirosis in veterinary practitioners, pet owners and public health institutions.

Prospective studies on leptospirosis have been performed in humans [24,25], however in domestic dogs these studies are limited. In Switzerland, 298 canines were diagnosed with leptospirosis in a veterinary hospital during 10 years, with an incidence rate of 5.88 diagnosed cases per 100.000 dogs per year, with an increase of new cases through years [26]. This represents the incidence of clinical leptospirosis, which is different than the incidence of anti-*Leptospira* antibodies. This was estimated in two studies in Brazil: in the first, out of 228 dogs sampled in 2009, ninety were re-sampled in 2010 and 35 animals were serological reactors to MAT with an incidence of 28.9% [27]. In the second study, the seroincidence rate of anti-*Leptospira* antibodies ranged from 6.0% in July-November 2010 to 15.3% in April-July 2011 [28].

In the present study, four dogs with serological conversion were of mixed breed, 4 were of small size, 5 were males, all were adults (from 1 to 12 years old) and none had clinical signs of leptospirosis (Table 2). In general, cohort studies on leptospirosis in canines determine few demographic characteristics of the animals with serological conversion. In one study [27], most of the new cases of the disease were males, which coincides with the findings of our study. However, in the survey of Major et al [26], the highest rate of serological conversion was observed in dogs less than 1 year old, which contrast with this study, differences that can be due to the characteristics of the sampled animals.

In the first sampling in the present study, serological reactions to a wide number of serovars were found, which is frequent in serological surveys of *Leptospira* infection [13,29]. The reactions by *Leptospira* specie, serovar and serogroup were the following: *L. borgpetersenii* serovar Ballum (5/25, 20.0%) (Serogroup Ballum), *L. interrogans* serovars Canicola (4/25, 16.0%) (Serogroup Canicola), Icterohaemorragiae (3/25, 12.0%) (Serogroup Icterohaemorragiae), Pyrogenes (3/25, 12.0%) (Serogroup Pyrogenes), Pomona (2/25, 8.0%) (Serogroup Pomona), Autumnalis (2/25, 8.0%) (Serogroup Autumnalis), Javanica (1/25, 4.0%) (Serogroup Javanica), Bataviae (1/25, 4.0%) (Serogroup Bataviae) and *L. kirchneri* serovar Grippotyphosa (1/25, 4.0%) (Serogroup Grippotyphosa). Since the most frequent serovars detected were Ballum and Canicola, the contact with rats, mice or other rodent species, as well as infected dogs could be the potential infection source for seropositive animals considering the maintenance hosts for those serovars [30,31].

Coagglutinations were detected in 12.0% (3/25) of seropositive dogs with the following serovars: Canicola/Icterohaemorragiae (coagglutination 1), Pyrogenes/Tarassovi/Wolfii (coagglutination 2) and Pyrogenes/Wolfii/Australis/Cynopteri/Patoc (coagglutination 3), which suggest current or recent infections, in which is not possible to indicate with accuracy the infecting serogroup, because antibodies against genus *Leptospira* appear earlier than serogroup-specific antibodies [20].

The dogs with serological conversions reacted to *L. interrogans* serovars Icterohaemorragiae (2/6, 33.3%) (Serogroup Icterohaemorragiae), Hardjo, (serogroup Sejroe), Pomona (serogroup Pomona), Autumnalis (serogroup Autumnalis) (1/6, 16.7% each one) and *L. borgpetersenii* serovar Ballum (serogroup Ballum) (1/6, 16.7%) (Table 2). No Coagglutinations were found. Different rodent such as rats and mice are the maintenance hosts for serovars Icterohaemorragiae, Autumnalis and Ballum [30] and the contact with these reservoir species is probably the origin of the serological reactions. Regarding seropositivity to serovar Hardjo, the reservoir hosts for this serovar are mainly cattle [9]; since the origins of the sampled animals in this study were urban areas (cities of Valdivia and Paillaco) in Los Ríos Region in southern Chile, the reason for the reactivity to serovar Hardjo is unclear. However, travels of dogs to other cities, rural areas or farms could be possible; therefore we cannot rule out the contact of the pets with reservoir hosts for this serovar.

The first sampling period was performed between January and November and the second was 6- 9 months later. Different studies have determined that leptospirosis in dogs is associated with the average rainfall registered 3 months before the diagnosis [32]. In southern Chile, the climate is mostly rainy, but both samplings included rainy seasons and drier weather. The specific association about cases of leptospirosis and climate in Chile is beyond the scope of this study, but it should be addressed in future research.

The antibody titers detected in MAT seropositive dogs ranged between 1:100 and 1:800 in both samplings. In the first sampling, the majority of serological reactors had low titers (1:100: n=14, 56.0% and 1:200: n=6, 24.0%) and few had higher titers (1:400: n=3, 12.0% and 1:800: n=2, 8.0%). Coagglutinations showed titres of 1:100 and 1:200. In the second sampling, most of the antibody titers were 1:100 (n=5, 83.3%) and one sample had a titer of 1:800 (16.7%). These low antibody titers may indicate recent exposures to the bacterium, but according [7], serological titers in response to *Leptospira* infection can decrease rapidly, consequently low titers may also represent ancient infections. It is known that dogs infected with serovar Canicola can be infected and shed the bacterium even with titers lower than 1:400 [30]. Furthermore, the renal carriage of *Leptospira* in canines and the circulation of the same serogroups detected in human cases of leptospirosis were recently confirmed in one study [33]. Is important to relate MAT results with clinical history of the patients for a properly interpretation [34]. In case of low antibody titers, bacteriological or molecular diagnostic tests are required to determine the urinary shedding and to infer the role of domestic dogs in the transmission of the bacterium to humans or other animals.

Since all samples from both samplings were analyzed with a panel of 6 serovars and only 26 samples from the first sampling were also tested with a panel of 12 different serovars, it is possible that some serological reactive dogs were not diagnosed. Besides, the reduced number of paired-serum samples could limit the identification of animals with serological conversion to anti-*Leptospira* antibodies. These issues are limitations of this research. Despite this, the present study constitutes one of the first prospective surveys to infer the dynamic of seropositivity to

Leptospira in domestic dogs in Chile.

CONCLUSION

The relatively high rate of serological conversion estimated in this study suggests that the exposure to *Leptospira* in pet dogs in southern Chile is present, with a possible endemic presentation of the seropositivity. Is important to take preventive measures such as vaccination and to reduce the exposition of pet dogs to reservoirs of the bacteria, as well as raise the awareness on leptospirosis among veterinary practitioners and pet owners given the zoonotic potential of the infection. The close contact between people and their pets increases the risk of transmission and needs special concern and attention. Additionally, the development of a surveillance system for zoonotic diseases in companion animals must be considered by veterinary and public health institutions in Chile.

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