Updates in Public Health and Preventive Medicine

Research Article Open Access

Similar Prevalence of Anti-*Leptospira* Antibodies in Domestic Dogs from Urban and Rural Areas in Southern Chile: A Public Health Concern [Version 1, 1 Approved with Reservations]

L Azócar-Aedo[1]*, G Monti[2] and R Jara[3]

[1]Graduate School, Faculty of Veterinary Sciences, Universidad Austral de Chile, Chile

[2]Preventive Veterinary Medicine Department, Faculty of Veterinary Sciences, Universidad Austral de Chile, Chile

[3]Biochemistry and Microbiology Department, Faculty of Sciences, Universidad Austral de Chile, Chile

***Corresponding author:** L Azócar-Aedo, Graduate School, Faculty of Veterinary Sciences, Universidad Austral de Chile, Campus Isla Teja, P.O. Box 567, Valdivia, Chile, Email: luciaazocaraedo@gmail.com

Original Submission

Received: January 30, 2017
Accepted: February 26, 2017
Published: March 05, 2017
Last Updated: April 05, 2017

Open Peer Review Status: 1 Approved with Reservations

How to cite this article: L Azócar-Aedo, G Monti, R Jara. Similar Prevalence of Anti-*Leptospira* Antibodies in Domestic Dogs from Urban and Rural Areas in Southern Chile: A Public Health Concern [Version 1, 1 Approved with Reservations]. Updates Public Health Prev Med. (2017) 1: 3.1

Acknowledgments: We thank to Victoria Calderón, Karin Venegas, Asunción Gallardo, Lizbeth Rogel, Edgardo Jara, Pilar Cerna, Hilary Soto, Karen Berrocal, Carlos Contreras and Veterinarians without Borders. This study was funded by CONICYT scholarship to support doctoral theses (scholarship #24121542), Chile 2012; FONDECYT regular project #1101020), Chile 2010; MECESUP AUS 1203 project, Chile 2013 and Graduate School, Faculty of Veterinary Sciences, Universidad Austral de Chile. L. Azócar-Aedo was supported by a scholarship from CONICYT, Chile.

Abstract

A cross-sectional study in domestic dogs was conducted in southern Chile aimed to estimate the Apparent Prevalence (AP) of anti-Leptospira antibodies in dogs from urban and rural environments using a frequentist method and, to model the True Prevalence (TP) using a Bayesian approach.

Blood samples from 220 urban dogs and 267 rural dogs were taken. Seropositivity was diagnosed using Microscopic Agglutination Test (MAT). A frequentist approximation was used to estimate the AP and a Bayesian approach was applied for TP estimations, considering a prior for high sensitivity and specificity in MAT (model 1), and a prior of low sensitivity and high specificity in the diagnostic test (model 2).

The APs of anti-*Leptospira* antibodies were 10.0% and 9.4% in urban and rural dogs, respectively. In the Bayesian models, TPs estimations were comparable in animals from urban and rural environments. In conclusion, the presence of anti-*Leptospira* antibodies is widespread in dogs of urban and rural environments in southern Chile with similar prevalences. Considering the zoonotic potential of *Leptospira* and the close contact of humans with their pets, preventive measures must be taken in both settings and an increase in awareness about leptospirosis among public health institutions and canine clinical practitioners is advisable.

Keywords

Anti-*Leptospira* Antibodies; Prevalence; Dogs; Urban Areas; Rural Areas; Zoonosis; Publi Health

Introduction

Leptospirosis is probably one of the most widespread and prevalent zoonotic disease in the world, having a great impact in veterinary medicine and public health [1,2]. It is an infection caused by a motile spirochaetal bacterium of the genus *Leptospira* [3].

Leptospirosis has an important clinical presence in canine medicine [4]. Animals become infected by the exposure of intact mucous membranes or abraded skin to contaminated urine or urine-contaminated soil or water [4-6]. The severity of the clinical signs depends on the age and immune status of the host and the virulence of the infecting serovar [7]. In general, suspected cases of canine leptospirosis show clinical signs of hepatic or renal failure, uveitis, acute febrile illness or pulmonary hemorrhage [8]. The disease could be life-threating for some dogs, but many cases are subclinical [9, 10]. Healthy dogs can shed leptospires through urine, posing a zoonotic risk [1, 12]. Considering the variability of the clinical signs, the prevalence of *Leptospira* infection in canines may be underestimated, which added to the lack of diagnostic capacity in many endemic regions, it contributes to the sub diagnosis and neglection of the disease [13, 14].

The distribution patterns leptospirosis in canine populations can vary greatly [15]. The main factors in the emergence of the infection are the interaction among animal reservoirs, the bacterium, animal susceptibility and the environment in which they coexist [16]. Therefore, infection and seropositivity are associated with a spectrum of environmental settings and exposures that are found in both urban and rural areas [17, 18].

Recently, a re-emergence of *Leptospira* infection has raised concern about the disease among pet populations in different geographic areas worldwide [19, 20]. Urban environments are important places where transmission of leptospirosis can occur [21]. The growth of urban centers, which facilitates the proliferation of reservoir hosts such as rodents, is an important factor to consider in the occurrence of the infection [22]. Stray dogs that roam free in urban areas are vulnerable to the disease, and dogs in suburban areas are also at risk due to probable contact with wildlife [23, 24]. On the other hand, dogs living in rural areas could be at risk of L*eptospira* infection considering that rural environments are the habitat of livestock, rodents and small mammals, which are frequent reservoirs for leptospires [22, 25, 26, 27].

In South America, cross-sectional studies of *Leptospira* infection in domestic dogs, assessing the prevalence in urban and rural areas are very limited, with the exception of studies carried out in Perú [28], Colombia [29, 30] and Brazil [31]. In Chile, the research on leptospirosis in dogs is also limited, but Lelu et al [32] performed a cross- sectional study in canines sampled in farms, rural villages and urban slums in Los Rios region, in which the serologic profiles of *Leptospira* were characterized, as well as factors influencing distinct observed patterns. However, how different is the magnitude of the prevalence between urban and rural animals and how the prevalence can change regarding the performance of the diagnostic test used remains unknown. Considering these issues, the aims of this study were: 1) to estimate the Apparent Prevalence of anti-*Leptospira* antibodies in dogs from urban and rural areas using a frequentist method and, 2) to model the True Prevalence of Anti-*Leptospira* antibodies using a Bayesian approach considering two models of the diagnostic performance of the Microscopic Agglutination Test.

Material and Methods

Study Area

The study was performed in urban and rural areas in Los Ríos and Los Lagos regions in southern Chile. We defined urban areas as territory areas located within the urban boundary, with more than 2000 inhabitants, with a set of concentrated housing [33, 34]. Rural areas were defined as territory areas located outside the urban boundary, or territory with a population below to 150 inhabitants per square kilometre [34, 35].

The urban areas were the data were collected were the cities of Valdivia and Paillaco located in Los Ríos region and the cities of San Pablo and Osorno located in Los Lagos region in southern Chile. The rural areas where the data were collected were dairy farms situated between Panguipulli (Los Ríos region) and Los Muermos (Los Lagos region). Some characteristics of the study area are described by Azócar-Aedo et al [36].

Sample Size Estimation

For urban areas, a population of 44.827 dogs was estimated with a human: dog ratio of 6.2 for cities and 5.3 for small towns [37]. Considering the human population of the cities of Valdivia, Osorno, Paillaco and San Pablo recorded in the 2002 Chilean Census [38], with an expected prevalence of 14.8% [39], a precision of 5% and a confidence level of 95%, the calculated sample size was 194 dogs, but finally 220 samples were taken in total. The number of samples collected in each city are detailed in Table 1.

Table 1: Apparent Prevalence (AP) of anti-Leptospira antibodies in domestic dogs from urban and rural areas obtained using a frequentist approximation.

Area	Categories	No. of dogs sampled	No. of MAT seropositive dogs	% of AP (95% CI)	
Urban	Overall	220	22	10.0	(6.4-14.7)
	Valdivia	35	4	11.4	(3.2-26.7)
	Osorno	29	6	20.7	(8.0-39.7)
	Paillaco	95	6	6.3	(2.4-13.2)
	San Pablo	61	6	9.8	(3.7-20.2)
Rural	Overall	267*	25	9.4	(6.2-13.5)
	Los Rios region	209*	18	8.6	(5.2-13.3)
	Los Lagos region	50*	5	10.0	(3.3-21.8)

* In rural areas, in 8 samples with 2 positive dogs, the region of origin was not recorded.

In rural areas in Chile, there are no data regarding the human: dog ratio, therefore, we considered the information provided by a canine census [40], in which an approximate canine population of 215 animals in 30 dairy farms located in Los Ríos region were recorded (7.2 dogs/dairy farm). In the present study, 110 dairy farms from Los Ríos and Los Lagos regions were included. Considering the ratio of dogs/farm aforementioned, a canine population of 792 animals within the 110 farms was estimated. Thus, with an expected prevalence of 37.0% [41], a precision of 5% and a confidence level of 95%, the calculated sample size was 247. Finally, 267 samples were taken in total. The number of samples taken in each region are listed in Table 1.

Study Design and Population Surveyed

The study design was cross-sectional. Between January 2011 and September 2012, blood samples were collected. The inclusion criteria was male and female owned domestic dogs, older than two months of age, of different breeds from urban areas or rural settings and without vaccination against leptospirosis.

In urban areas, dogs were selected randomly from four veterinary clinics (veterinary clinics who agreed to participate in the study voluntarily); they were recruited for the study during home visits or in a veterinary neutering campaign. The sample included animals attending veterinary clinics for different reasons and healthy dogs.

In rural areas, the animals were sampled during farm visits, in which all available dogs were recruited and sampled for study. The farms were selected randomly from a list available at Preventive Veterinary Medicine Department, Faculty of Veterinary sciences, Universidad Austral de Chile.

The owners and practitioners were informed about the aims of the study and they agreed to participate in the survey voluntarily.

Field and Laboratory Procedures

Blood samples (1-2 ml) were collected by venipuncture. The presence of anti-*Leptospira* antibodies was detected using the Microscopic Agglutination Test (MAT). A total of 487 samples (220 from urban dogs and 267 from rural dogs) were analyzed with six *Leptospira* serovars in agreement with published methods [42]. Live cultures of strains representative of L. *interrogans* serovars Hardjo, Pomona, Canicola, Icterohaemorragiae and Autumnalis and *L. borgpetersenii* serovar Ballum were used. The reference strains for each serovar were: Hardjoprajitno (Hardjo), Pomona (Pomona), Hond Utrecht IV (Canicola), Verdun (Icterohaemorragiae), Akiyami A (Autumnalis) and S102 (Ballum).

The criteria to consider a sample with anti-*Leptospira* antibodies was a single titre of 1:100 or greater in MAT [43].

Ethics Statement

The methods for animal handling and blood extraction used in this study were approved by the bioethics committee at the Universidad Austral de Chile (certification number #10-2012).

Frequentist and Bayesian Approximation of the Prevalence

The Apparent Prevalence (AP) of anti-*Leptospira* antibodies for canines of urban and rural areas was estimated based on MAT results with a frequentist approximation using a published methodology [44]. The 95% Confidence Intervals (95% CI) were calculated using a method by Pretie and Watson [45], which consist in adding to, and subtracting from the calculated prevalence, the z value of its standard error. The possible differences between prevalences in urban and rural environments were assessed using Chi square test for statistical significance ($p<0.05$) with EpiInfo version 6.04.

A Bayesian approach was used to obtain the True Prevalence (TP) of anti-*Leptospira* antibodies considering the uncertainty of the diagnostic performance of MAT (Sensitivity (Se) and Specificity (Sp)). This uncertainty was modeled using the following independent beta prior distributions: *Se~Beta(aSe,bSe) Sp~Beta(aSp,bSp),* in which the prior parameters of the *beta* distributions were estimated based on the most likely (modal) prior value of the parameter and an upper or lower percentile for the parameter [46]. In the beta prior distributions, the acronym aSe, represent the minimum value for the Se and bSe is the maximum value of Se. Otherwise, the acronym *aSp*, is the minimum value for the Sp and *bSp* indicate the maximum value for the Sp.

Two models were considered according to bibliographic information available about the diagnostic performance of MAT: a prior distribution for a high Se (98.2%) and Sp (96.4%) (model 1) [47], and a prior for a low Se (49.8%) and high Sp (98.8%) (model 2) [48]. In the Bayesian models, the Brooks-Gelman-Rubin (BGR) statistic was used to determine the convergence values of the models, which was assessed comparing the estimated between-chains and within-chain variances for each model parameter. An approximate convergence was detected if the upper limit of the BGR statistic value was close to 1 [49, 50]. The median of the posterior distribution and the 95% Credibility Intervals (95% CrI) of the parameters of interest were also estimated. These analyses were performed using R version 3.0 (Package Prevalence) [51].

Results

Frequentist Approximation of AP of Anti-*Leptospira* Antibodies in Dogs from Urban and Rural Areas

In urban dogs, the overall AP of anti-*Leptospira* antibodies was 10.0% (95% CI=6.4-14.7). MAT serological reactor dogs were found in all cities included in the study. The APs for each city were Valdivia: 11.4% (95% CI=3.2-26.7), Osorno: 20.7% (95% CI=8.0-39.7), Paillaco: 6.3% (95% CI=2.4-13.2) and San Pablo: 9.8% (95% CI=3.7-20.2) (Table 1).

In rural dogs, the AP of anti-*Leptospira* antibodies was 9.4% (95% CI=6.2-13.5). The AP in dairy farms in Los Ríos Region was 8.6% (95% CI=5.2-13.3) and in dairy farms in Los Lagos Region was 10.0% (95% CI=3.3-21.8) (Table 1).

No statistically significant differences were observed in APs between urban and rural environments.

Bayesian Approximation of TP of Anti-Leptospira Antibodies in Domestic Dogs from Urban and Rural Areas

Table 2 shows the estimates of the TPs and the 95% Credibility intervals (CrI) using the Bayesian approach. The TPs varied between the models 1 and 2. In urban dogs, the TPs ranged from 7.0% (model 1) to 12.1% (model 2) and from 6.2% (model 1) to 11.1% (model 2) in rural dogs. In models 1 and 2, the prevalence estimates were similar between animals from urban and rural environments. All BGR values were close to 1 or not substantially higher than 1.

Table 2: True Prevalence (TP) of anti-Leptospira antibodies in domestic dogs from urban and rural areas obtained using a Bayesian approximation.

Models	Categories	% of TP (95% CrI)	
Model 1	Urban areas	7.0	(1.7-13.1)
	Rural areas	6.2	(1.4-11.7)
Model 2	Urban areas	12.1	(0.9-25.2)
	Rural areas	11.1	(0.8-23.0)

Discussion

The present study estimated the AP and TP for *Leptospira* in dogs from urban and rural areas in Los Ríos and Los Lagos regions in southern Chile. The main finding was the detection of similar prevalences of anti-*Leptospira* antibodies in pet dogs in both environments.

In dogs from urban areas, the AP was 10.0%. This coincides with studies in New Zealand (12.0%) [52] and Thailand (11.0%) [53], but it is not consistent with other surveys results in urban animals in Colombia (20.5%) [29], Brazil (27.8%) [31], Egypt (58.3%) [54] and India (71.1%) [55]. Rural dogs in this study had an estimated AP of 9.4%, which is similar to a report in Greece (11.4%) [56]. However, higher APs have been reported in rural dogs in Trinidad and Tobago (20.4%) [57], Colombia (35.2%) [30], Iran (36.9%) [58] and Thailand (57.5%) [26].

Some cross-sectional studies on *Leptospira* in dogs had been performed earlier in Chile, but they were restricted to one city and areas of reduced extension within a particular geographic area. Similar APs with the present study were observed in urban dogs [39]. However, contrasting results were recorded in another survey [59]. This variability in leptospiral seroprevalence within the same country was also noted in Brazil, where dissimilar frequencies were observed in different locations: 7.1% in Bahia [60] and 27.3% in Rondonia [23]. The use of different diagnostic tests, survey periods (seasons of the year), characteristics of the sampled animals, sampling designs, distinct recruitment methods of the animals in the sampling, or cut-off values to consider an animal as serological reactor in the diagnostic test can result in differences in the prevalence among countries or within a country [61].

Recently, Lelu et al [32] performed a cross-sectional study on canine leptospirosis in urban slums in Los Ríos region in Chile, in which an AP of 45.1% was reported. This result contrast with the overall AP in urban areas estimated in the present study (10.0%). Lelu et al [32] defined urban slums as informal settlements in the outskirts of a major city, characterized by substandard housing. In our study, the sampling was performed in Los Ríos and Los Lagos regions in urban areas in general, not in urban slums. In humans, the presence of leptospirosis in urban slums has been demonstrated [62, 63, 64, 65], which could be related to the habitat in close proximity to environmental sources of leptospires, such as open sewers, flood areas, garbage and the presence of rodents [66]. Considering the proximity of humans and dog populations, these conditions could also influence the prevalence of the disease in canines and, it explain the high prevalence reported in the survey of Lelu et al [32] compared with our study.

Regarding rural areas, in the study performed in Chile by Lelu et al [32], dogs were sampled in farms and rural villages in Los Ríos region. In the present study, the rural areas were dairy farms located in Los Rios and Los Lagos regions. As we do not sampled in rural villages, we can only compare our results with the AP obtained by that study in farms. That AP was 10.9%, which was similar to the AP reported to our study (9.4%).

In veterinary medicine, the Bayesian approach for the estimation of the TP has been used in prevalence studies in different diseases such as paratuberculosis, foot-and-mouth disease or pathogens such as *Yersinia* [67, 68, 69], but it has not been applied frequently to *Leptospira* infection or in pet diseases. In this analysis, even in the model with low Se for the diagnostic test (model 2), a TP of 12.1% and 11.1% were estimated in urban and rural dogs, respectively, which confirms the presence of anti-*Leptospira* antibodies in dogs in the study area and the exposure to the bacteria.

In Bayesian models 1 and 2, the TPs were comparable in dogs from urban and rural areas, which was also observed in the APs calculated with the frequentist approximation. This suggests that the presence of anti-*Leptospira* antibodies is widespread among domestic dogs from urban and rural environments in the study area with similar prevalences, which represent an important public health concern, considering the zoonotic potential of canine leptospirosis and the close contact of humans with their pet dogs. A higher prevalence of anti-*Leptospira* antibodies could be expected in rural areas, taking into account environmental and lifestyle conditions in rural environments, such as the presence of livestock or rodents [22, 27]. However, the similar APs and TPs recorded in this study highlight the fact that the conditions for transmission of leptospires in companion animals do exist in both, urban and rural areas, emphasizing the need to raise the awareness about the disease and to take preventive measures.

It is important to note that although MAT can detect seropositivity to different leptospiral serogroups [18], serological reactions may be no detectable if a representative strain of a serogroup is not present in the panel used [70]. Since the MAT serovar panel included six different serovars, it is possible that some seropositive dogs were not detected and this could be a limitation for the present study.

Conclusions

This study demonstrates that urban and rural domestic dogs in southern Chile are exposed to leptospires and the presence of antibodies is widespread between urban and rural areas with similar prevalences, which represent a public health concern. These findings allows us to formulate some recommendations to prevent the disease, such as the provision of information to dog owners about the epidemiological characteristics of *Leptospira* infection, which must be carried out in urban and rural settings. Moreover, an increase in the awareness in public health institutions and in canine clinical practitioners must be considered to reduce the exposure to *Leptospira spp* in pet dog populations living in urban and rural environments.

References

1. Levett P. Leptospirosis. Clin Microbiol Rev. 2001; 14: 296-326.
2. World Health Organization (WHO). Report of the Second Meeting of the Leptospirosis Burden Epidemiology Reference Group. Geneva, Switzerland. 2011.
3. Bharti A, Nally J, Ricaldi J, Mathias M, Diaz M, et al. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis. 2003; 3: 757-771.
4. Ananda KJ, Suryananarayana T, Sharada P, D´Souza P. Diagnosis and treatment of leptospirosis in a dog. A case report. Vet World. 2008; 1: 278-279.
5. Ko A, Goarant C, Picardeau M. Leptospira: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol. 2009; 7: 736-747.
6. Goldstein R. Canine leptospirosis. Vet Clin Small Anim. 2010; 40: 1091-1101.
7. Greene C, Sykes J, Brown C, Hartmann K. Leptospirosis. In: Greene C, editor. Enfermedades infecciosas del perro y el gato. Buenos Aires: Intermédica. 2006; 448-463.
8. Sykes J, Hartmann K, Lunn K, Moore G, Stoddard R, et al. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. J Vet Intern Med. 2011; 25: 1-13.
9. Katrin H, Graig E. Diseases caused by systemic bacterial infection. In: Ettinger S, Feldmann J, editors. Textbook of veterinary internal medicine. Philadelphia: Elsevier Saunders. 2005; 616-619.
10. Stokes J, Kaneene J, Schall W, Kruger J, Miller R, et al. Prevalence of serum antibodies against six Leptospira serovars in healthy dogs. J Am Vet Med Assoc. 2007; 230: 1657-1664.
11. Rojas P, Monahan A, Schuller S, Miller S, Markey B, et al. Detection and quantification of leptospires in urine

of dogs: a maintenance host for the zoonotic disease leptospirosis. Eur J Clin Microbiol Infect Dis. 2010; 10: 1305-1309.

12. Llewellyn J, Krupka-Dyachenko I, Rettinger A, Dyachenko V, Stamm I, et al. Urinary shedding of leptospires and presence of Leptospira antibodies in healthy dogs from Upper Bavaria. Berl Munch Tierarztl Wochenschr. 2016; 129: 251-257.
13. Smythe L. Leptospirosis worldwide. Wkly Epidemiol Rec. 1999; 76: 109-116.
14. Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clin Microbiol Infect. 2011; 17: 494-501.
15. Jimenez-Coello M, Vado-Solis V, Cárdenas-Marrufo, Rodríguez-Buenfil C, et al. Serological survey of canine leptospirosis in the tropics of Yucatan, Mexico using two different tests. Acta Trop. 2008; 106: 22-26.
16. Lau CL, Smythe LD, Craig SB, Weinstein P. Climate change, flooding, urbanisation and leptospirosis: fuelling the fire? Trans R Soc Trop Med Hyg. 2010; 104: 631-638.
17. Maciel EA, de Carvalho AL, Nascimento SF, de Matos RB, Gouveia EL, et al. Household transmission of leptospira infection in urban slum communities. PLoS Negl Trop Dis. 2008; 2: e154.
18. Picardeau M. Diagnosis and epidemiology of leptospirosis. Med Mal Infect. 2013; 43: 1-9.
19. Major A, Schweighauser A, Francey T. Increasing incidence of canine leptospirosis in Switzerland. Int J Environ Res Public Health. 2014; 11: 7242-7260.
20. Damborg P, Broens E, Chomel B, Guenther S, Pasmans F, et al. Bacterial zoonoses transmitted by household pets: state of the art and future perspectives for targeted research and policy actions. J Comp Path. 2015; 155: S27-S40.
21. Cachay ER, Vinetz JM. A global research agenda for leptospirosis. J Postgrad Med. 2005; 51: 174-178.
22. Ward MP, Guptill LF, Prahl A, Wu CC. Serovar-specific prevalence and risk factors for leptospirosis among dogs: 90 cases (1997-2002). J Am Vet Med Assoc. 2004; 224: 1958-1963.
23. Aguiar D, Cavalcante G, Marvulo M, Silva J, Pinter A, et al. Risk factors associated with anti-Leprospira spp antibodies occurrence in dogs from Monte Negro County, Rondonia, Brazilian Western Amazon. Arq Bras Med Vet Zootec. 2007; 59: 70-76.
24. Querino A, Delbem A, Oliveira R, da Silva S, Muller E, et al. Risk factors associated with leptospirosis in dogs in Londrina city-PR. Semina Ciencias Agrarias. 2003; 24: 27-34.
25. Ratnam S. Leptospirosis: an Indian perspective. Indian. J Med Microbiol. 1994; 12: 228-239.
26. Niwetpathomwat A, Asarasakorn S. Preliminary investigation of canine leptospirosis in a rural area of Thailand. Medycyna Wet. 2007; 63: 59-61.
27. Richardson DJ, Gauthier JL. A serosurvey of leptospirosis in Connecticut peridomestic wildlife. Vector Borne Zoonotic Dis. 2003; 3: 187-193.
28. Siuce J, Calle S, Pinto C, Pacheco G, Salvatierra G. Identification of pathogenic Leptospira serogroups in domestic dogs. Rev Inv Vet Perú. 2015; 26: 664-675.
29. Silva-Molano R, Castro F, Montoya J, Loaiza-Echeverri A. Frequency study of canine leptospirosis in Manizales-Colombia, with the aid of Microscopic Agglutination Test (MAT). Vet Zootec. 2008; 2: 35-39.
30. Calderón A, Rodríguez V, Máttar S, Arrieta G. Leptospirosis in pigs, dogs, rodents, humans, and water in an area of the Colombian tropics. Trop Anim Health Prod. 2014; 46: 427-432.
31. Bier D, Shimakura S, Morikawa S, Ullmann V, Kikuti L, et al. Spatial analysis of the risk of canine leptospirosis in the Vila Pantanal, Curitiba, Paraná, Brazil. Pesq Vet Bras. 2013; 33: 74-79.
32. Lelu M, Muñoz-Zanzi C, Higgins B, Galloway R. Seroepidemiology of leptospirosis in dogs from rural and slum communities of Los Rios Region, Chile. BMC Vet Res. 2015; 11: 31.
33. INE (Instituto Nacional de Estadísticas, Chile). Census glossary. National Institute of Statistics, Government of Chile. 2002.
34. MINVU (Ministerio de Vivienda y Urbanismo, Chile). General Ordinance of Urbanism and Constructions, Ministry of Housing and Urbanism, Government of Chile. 2009.
35. OCDE. The New Rural Paradigm: Policies and Governance. OECD Rural Policy Reviews. Organisation for Economic Co-Operation and Development. Paris, France. 2006.
36. Azócar-Aedo L, Monti G, Jara R. Leptospira spp in domestic cats from different environments: prevalence of antibodies and risk factors associated with the seropositivity. Animals. 2014; 4: 612-626
37. Acosta-Jamett G, Cleaveland S, Cunningham A, Bronsvoort BM de C. Demography of domestic dogs in rural and urban areas of the Coquimbo region of Chile and

implications for disease transmission. Prev Vet Med. 2010; 94: 272-281.

38. INE (Instituto Nacional de Estadísticas, Chile). Cuadros censales. 2016. Available at: http://espino.ine.cl/CuadrosCensales/apli_excel.asp

39. Silva R, Riedemann S. Frequency of canine leptospirosis in dogs attending veterinary practices determined through microscopic agglutination test and comparison with isolation and immunofluorescence techniques Arch Med Vet. 2007; 39: 269-274.

40. Ojeda X. Caracterización de la población canina rural (Canis lupus familiaris) en predios lecheros de la zona sur de Chile. Veterinarian Thesis, Universidad Austral de Chile, Chile. 2008.

41. Zamora J, Rojas X, Kruze J, Vallejos N. Investigación serológica de leptospirosis y brucelosis canina en el área rural de la comuna de Los Ángeles, Bío-Bio. Arch Med Vet. 1971; 3: 5-10

42. Faine S. Guidelines for the control of leptospirosis. World Health Organization. WHO Offset Publ. 1982; 67: 161.

43. Alton GD, Berke O, Reid-Smith R, Ojkic D, Prescott JF, et al. Increase in seroprevalence of canine leptospirosis and its risk factors, Ontario 1998-2006. Can J Vet Res. 2009; 73: 167-175.

44. Fletcher R, Fletcher S. Frequency: In: Fletcher R, Fletcher S, editors. Clinical epidemiology: the essentials. Philadelphia: Lippincott Williams & Wilkins. 2005; 62.

45. Pretie A, Watson P. Statistics for veterinary and animal science. Oxford: Blackwell Publishing. 1999.

46. Suess EA, Gardner IA, Johnson WO. Hierarchical Bayesian model for prevalence inference and determination of a country's status for an animal pathogen. Prev Vet Med. 2002; 55: 155-171.

47. Bajani MD, Ashford DA, Bragg SL, Woods CW, Aye T, et al. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. J Clin Microbiol. 2003; 41: 803-809.

48. Limmathurotsakul D, Turner E, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol Y, et al. Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. Clin Infect Dis. 2012; 55: 322-331.

49. Gelman A, Rubin D. Inference from iterative simulation using multiple sequences. Stat Sci. 1992; 7: 457-511.

50. Brooks S, Gelman A. General methods for monitoring convergence of iterative simulations. J Comp Grap Stat. 1997; 7: 434-455.

51. Speybroeck N, Devleesschauwer B, Joseph L, Berkvens D, et al. Misclassification errors in prevalence estimation: Bayesian handling with care. Int J Public Health. 2013; 58: 791-795.

52. O'Keefe JS, Jenner JA, Sandifer NC, Antony A, Williamson NB, et al. A serosurvey for antibodies to Leptospira in dogs in the lower North Island of New Zealand. N Z Vet J. 2002; 50: 23-25.

53. Meeyam T, Tablerk P, Petchanok B, Pichpol D, Padungtod P, et al. Seroprevalence and risk factors associated with leptospirosis in dogs. Southeast Asian J Trop Med Public Health. 2006; 37: 148-153.

54. Samir A, Soliman R, El-Hariri M, Abdel-Moein K, Hatem ME, et al. Leptospirosis in animals and human contacts in Egypt: broad range surveillance. Rev Soc Bras Med Trop. 2015; 48: 272-277.

55. Ambily R, Mini M, Joseph S, Krishna S, Abhinay G. Canine leptospirosis- a seroprevalence study from Kerala, India. Vet World. 2013; 6: 42-44.

56. Burriel AR, Dalley C, Woodward MJ. Prevalence of leptospira species among farmed and domestic animals in Greece. Vet Rec. 2003; 153: 146-148.

57. Adesiyun AA, Hull-Jackson C, Mootoo N, Halsall S, Bennett R, et al. Sero-epidemiology of canine leptospirosis in Trinidad: serovars, implications for vaccination and public health. J Vet Med B Infect Dis Vet Public Health. 2006; 53: 91-99.

58. Rad M, Zeinali A, Van Yousofi J, Tabatabayi A. Seroprevalence and bacteriological study of canine leptospirosis in Tehran and its suburban areas. Iran J Vet Res. 2004; 5: 2-10.

59. Tuemmers C, Luders C, Rojas C, Serri C, Espinoza R, et al. Prevalence of leptospirosis in vague dogs captured in Temuco city, 2011. Rev Chilena Infectol. 2013; 30: 252-257.

60. Oliveira Lavinsky M, Said RA, Strenzel GM, Langoni H. Seroprevalence of anti-Leptospira spp. antibodies in dogs in Bahia, Brazil. Prev Vet Med. 2012; 106: 79-84.

61. Azevedo SS, Batista CS, Vasconcellos SA, Aguiar DM, Ragozo AM. Seroepidemiology of Toxoplasma gondii and Neospora caninum in dogs from the state of Paraíba, Northeast region of Brazil. Res Vet Sci. 2005; 79: 51-56.

62. Kaur I, Sachdeva R, Arora V, Talwar V. Preliminary survey of leptospirosis amongst febrile patients from ur-

ban slums of East Delhi. J Assoc Physicians India. 2003: 5: 249-251.

63. Johnson MA, Smith H, Joeph P, Gilman RH, Bautista CT, et al. Environmental exposure and leptospirosis, Peru. Emerg Infect Dis. 2004; 10: 1016-1022.

64. Felzemburgh R, Ribeiro G, Costa F, Reis R, Hagan J, et al. Prospective study of leptospirosis transmission in an urban slum community: role of poor environment in repeated exposures to the Leptospira agent. PLoS Negl Trop Dis. 2014; 29: 154.

65. Hagan J, Moraga P, Costa F, Capian N, Ribeiro G, et al. Spatiotemporal determinants of urban leptospirosis transmission: four-year prospective cohort study of slum residents in Brazil. PLoS Negl Trop Dis. 2016; 15.

66. Sarkar U, Nascimento S, Barbosa R, Martins R, Nuevo H, et al. Population-based case-control investigation of risk factors for leptospirosis during an urban epidemic. Am J Trop Med Hyg. 2002; 66: 605-610.

67. Van Schaik G, Schukken YH, Crainiceanu C, Muskens J, VanLeeuwen JA. Prevalence estimates for paratuberculosis adjusted for test variability using Bayesian analysis. Prev Vet Med. 2003; 60: 281-295.

68. Goris N, Praet N, Sammin D, Yadin H, Paton E, et al. Foot-and-mouth disease non-structural protein serology in cattle: use of a bayesian framework to estimate diagnostic sensitivity and specificity of six ELISA tests and true prevalence in the field. Vaccine. 2007; 25: 7177-7196.

69. Vilar M, Ranta J, Virtanen S, Korkeala H. Bayesian estimation of the True Prevalence and of the diagnostic test sensitivity and specificity of entheropathogenic Yersinia in finish pigs serum samples. Biomed Res Int. 2015.

70. Safiullah S, Abu Saleh A, Munwar S. Laboratory methods for diagnosing leptospirosis: a review. Bangladesh. J Med Microbiol. 2009; 3: 39-43.