ANNA CECÍLIA TROLESI REIS BORGES COSTA

EPIDEMIOLOGY OF LEPTOSPIROSIS IN HUMANS AND UNOWNED DOGS: A ONE HEALTH APPROACH

Lavras - MG

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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-graduação em Ciências Veterinárias, área de concentração em Sanidade Animal e Saúde Coletiva, para obtenção do título de Mestre.

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Orientadora

Prof. Dr. Marcos Bryan Heinemann

Co-Orientador

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I dedicate to grandmother (in memory), thank you for being strong until the end.

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First and for most I thank God for the life that I have, the strength and grace to keep going even when there were difficulties, without Your grace I could not have done it!

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“Those that never change their mind never change anything!”

Winston Churchill

ABSTRACT

The focus of the present dissertation was to contribute to the knowledge about epidemiology of leptospirosis in humans and dogs. For this, a time series analysis of leptospirosis human cases from 2007 to 2019, a systematic review and a longitudinal study on the prevalence and seroprevalence of leptospirosis in unowned dogs were conducted. The time series analysis showed that human leptospirosis is endemic in Brazil, with a heterogeneous distribution among the Brazilian regions and most cases occurring at the raining season. Moreover, a robust forecast model for leptospirosis human cases in Brazil was built, exhibiting seasonality and successfully predicting the cases for the last six months of 2019. For the systematic review, the results pointed for a lack of well-developed cross-sectional studies among the recovered articles, preventing a meta-analysis to estimate the prevalence or seroprevalence of leptospirosis in street and sheltered dogs. On the other hand, the presence of *Leptospira* spp. or antibodies anti-*Leptospira* spp. in stray and sheltered dogs worldwide was fully observed. Finally, the preliminary results of the study on the prevalence of leptospirosis in dogs from a shelter revealed the absence of infection and a low seroprevalence of the disease (2.12%, 95%CI: 0.86 to 4.33) among the sampled dogs. In general, our results showed that leptospirosis as an important public and animal health issue in Brazil and worldwide, and a lack of robust epidemiological information on the disease among unowned dogs; but they also pointed to a controlled disease situation in a dog shelter in the municipality of Lavras, Minas Gerais state, Brazil.

Keywords: Zoonosis, *Leptospira* spp., public health, time series analysis, prevalence, seroprevalence, infectious disease.

RESUMO

O objetivo da presente dissertação foi contribuir para o conhecimento sobre a epidemiologia da leptospirose em humanos e cães. Para isso, foi realizada uma análise de série temporal de casos humanos de leptospirose de 2007 a 2019, uma revisão sistemática e um estudo longitudinal sobre a prevalência e soroprevalência da leptospirose em cães sem dono. A análise de séries temporais mostrou que a leptospirose humana é endêmica no Brasil, com distribuição heterogênea entre as regiões brasileiras e a maioria dos casos ocorrendo na época das chuvas. Além disso, um modelo robusto de previsão de casos humanos de leptospirose no Brasil foi construído, exibindo sazonalidade e prevendo os casos com sucesso para os últimos seis meses de 2019. Para a revisão sistemática, os resultados apontaram para a falta de estudos transversais bem desenvolvidos entre os artigos recuperados, impedindo uma meta-análise para estimar a prevalência ou soroprevalência da leptospirose em cães de rua e de abrigos. Por outro lado, a presença de *Leptospira* spp. ou anticorpos anti-*Leptospira* spp. em cães de rua e de abrigos em todo o mundo foi totalmente observado. Por fim, os resultados preliminares do estudo de prevalência de leptospirose em cães de abrigo revelaram ausência de infecção e baixa soroprevalência da doença (2,12%, IC 95%: 0,86 a 4,33) entre os cães amostrados. Em geral, nossos resultados mostraram que a leptospirose é um importante problema de saúde pública e para saúde animal no Brasil e no mundo, porém, além da falta de informações epidemiológicas robustas sobre a doença em cães sem dono, os resultados também apontaram para uma situação de doença controlada em um abrigo canino no município de Lavras, estado de Minas Gerais, Brasil.

Palavras-chave: Zoonoses, *Leptospira* spp., saúde pública, análise de séries temporais, prevalência, soroprevalência, doença infecciosa.

SUMMARY

1. GENERAL INTRODUCTION

The *Leptospira* spp. is a spirochete, Gram-negative bacterium that affects all mammals and humans, being responsible for causing leptospirosis, a neglected zoonotic disease. Among all mammals affected by leptospirosis, dogs are of particular interest due to their proximity to humans and thereby potential source of infection. The proximity between these two species continue even when dogs are abandoned, since they continue to live closely to the human population on the streets (unplanned) or in shelters (caretakers and potential adopters).

Considering that dogs can transmit leptospirosis to human and the disease could cause great losses (hospitalization, treatment, lost days of work) and deaths, to both populations (humans and animals), the complete understanding about the epidemiological situation of leptospirosis is fundamental to implement effective control and preventive measures against the disease. In this context, surveillance and control measures are tools that allow the identification of disease occurrence and the burden that it causes, as well as of any factor that influences its manifestation. It is important to use a holistic strategy, qualified as One Health approach, in which human and animal health are considered to deal with the losses caused by the disease and to implement successful control and preventive measures.

Therefore, the aim of this dissertation was to generate qualified information about leptospirosis in humans and unowned dogs to contribute to the comprehension of the epidemiological situation of the disease, using a One Health approach. To achieve this goal, we conduct a time series analysis of leptospirosis human cases in Brazil, proceed a systematic review on the prevalence of leptospirosis in street and sheltered dogs and performed a cross-sectional study to determine the prevalence and seroprevalence of leptospirosis in sheltered dogs from Lavras, Minas Gerais state, Brazil.

CHAPTER 1: Formatted according to the submission guidelines of Acta Tropica (Preliminary version)

Prediction of leptospirosis human cases in Brazil, 2007-2019.

Abstract

The aims of this study were (i) to perform a time series analysis to build a predict model of human leptospirosis cases and (ii) to estimate the disease incidence, mortality, and case fatality rates in Brazil. Data of human leptospirosis cases, deaths, and population, as well as precipitation data were recovered from different national databases. The annual incidence, mortality and case fatality rates of human leptospirosis and the average precipitation were calculated for the country and five Brazilian regions (North, Northeast, Midwest, Southeast and South). The time series analysis was performed using seasonal autoregressive integrated moving average models (SARIMA) for modeling. A forecast model was developed to predict the cases for the last six months of 2019. The results showed that human leptospirosis is endemic in Brazil, presenting a heterogeneous distribution among the regions, with most cases occurring at the raining season. The forecast model for human leptospirosis cases, with 95% prediction interval, predicted 1,731.11 cases for the last six months of 2019 and 1,326 cases were reported in this period. There was a positive correlation between precipitation and human leptospirosis cases (Spearman's $\rho = 0.39$, $p < 0.001$). In Brazil, considering the annual average for the evaluated period: the incidence of leptospirosis was 1,913 cases per 100,000 inhabitants, ranging from 0.44 per 100,000 (Midwest region) to 4.15 per 100,000 (South region); the leptospirosis mortality rate was 0.168 deaths per 100,000 inhabitants, ranging from 0.04 per 100.000 (Midwest region) to 0.25 per 100.000 (South region); and the leptospirosis case fatality rate was 8.83%, ranging from 6.10% (North region) to 12.43% (Southeast region). In conclusion, our results showed that the proposed predict model can be useful for the Brazilian

health system for planning leptospirosis surveillance and control actions, especially in the raining months when the disease incidence is higher; moreover health indicators revealed a non-uniform epidemiological situation of leptospirosis in the country.

Keywords: SARIMA, epidemiology, modeling, zoonosis, time series analysis.

1. Introduction

Leptospirosis is a zoonotic disease caused by bacteria of the genus *Leptospira* spp., a Gram-negative spirochete that affects a variety of mammals, including humans (Adler and de la Pena Moctezuma, 2010). The pathogen is transmitted to humans by contact with environment contaminated with urine from infected animals (Levett, 2001). The disease is present in all continents except Antarctica, affecting 1.03 million people with 58,900 deaths per year worldwide (Costa et al., 2015), causing a global burden of 2.50 Disability Adjusted Life Years (Torgerson et al., 2015).

The disease in humans usually exhibits unspecific symptoms (fever, myalgia and headache), making it difficult to differentiate from others diseases, such as dengue fever and influenza (Haake and Levett, 2015). In 10% of cases, the disease exhibits major complications, affecting the respiratory system with hemorrhages, kidney and liver failure caused by lesions on the parenchyma of the organ, which can lead to death (Cagliero et al., 2018).

In Brazil, human leptospirosis is endemic, being epidemic in the raining months (Souza et al., 2011), due to flooding that affect mostly low-income people that are agglomerated in slums (Maciel et al., 2008), poor sanitation conditions and constant presence of infected rodents, especially in capitals and metropolitan areas (Mwachui et al., 2015). Hospitalization costs associated with human leptospirosis, in 2007, were estimated in R$ 831,537.28 (U$ 146,868.03) per year and the years of potential life lost in 4 years per 100.000 population (Souza et al., 2011). Notification of human leptospirosis cases is mandatory in Brazil, generating monthly data of the disease (Brasil, 2016), which are available at Sistema de Informação de Agravos e Notificação – SINAN (Notification Disease Information System) (https://sinan.saude.gov.br/sinan/) (Brasil, 2019). A helpful tool to analyze this type of data is a time series analysis, by which it is possible to obtain a predict model for future cases. Indeed,

a mathematic model, along with the indicators of the disease by region can facilitate the early identification and control of the disease, as well as improve the prevention of cases in regions shown to be more affected by leptospirosis. Moreover, as leptospirosis is considered a neglected disease (Rodrigues, 2018), with low investment directed to prevention, it is important to consider that this knowledge also help to direct the financial support to where it is most needed, assisting a strategic planning of the Sistema Único de Saúde - SUS (Brazilian Universal Health System) (Brasil, 2013), especially in a scenery with several other demands which also require a portion of the available funds, already limited.

Therefore, the aims of this study were (i) to determine a mathematic model to predict future cases of human leptospirosis using a time series analysis, and (ii) to estimate the disease incidence, mortality and case fatality rates in Brazil, focusing on support the SUS in the planning the use of public resources.

2. Material and methods

2.1.Local and data source

Brazil is continental country with extension of 4 million square kilometers, located in South America, latitude between 5°16'20" north and 33°45'03" south, and longitude between 34°47'30" east and 73°59'32" west (IBGE, 2010). The national territory is divided in 26 states and a Federal District, distributed in five regions, North, Northeast, Midwest, Southeast and South.

The Brazilian climate, classified by the Köppen-Geiger system, showed Aw classification (Tropical climate with rain in the summer) for the majority of the country. The classification by regions was defined as follow: North region - Am [Tropical with annual precipitation average (APA) of >1,500 mm], Af (Tropical with precipitation all months) and Aw classifications; Northeast region - As (Tropical APA between 380 and 760 mm), BSh [Arid

with APA between 380 and 760 mm and annual average temperature (AAT) of >18 °C], Aw, Am and Af classifications; Southeast region - Aw, Am, Af, CFb (Temperate with precipitation in all year and in the hottest months temperature >10 °C to < 22 °C), CFa (Temperate with precipitation in all year in the hottest months temperature ≥ 22 °C), BWh (Arid, with APA of <200 mm and AAT of >18 °C), BSh (Arid, APA between 380 mm and 760 mm and AAT of >18 °C); South region - CFa, CFb, Af and Am classifications; and Mideast region - Aw and Am classifications (Dubreuil et al., 2018).

The data of monthly human leptospirosis confirmed cases and deaths according to state from January 2007 to December 2019, were obtained from SINAN (http://portalsinan.saude.gov.br/sinan) (Brasil, 2019). The monthly precipitation data according to state per month from January 2007 to December 2019, were obtained from the Instituto Nacional de Meteorologia – INMET (Nacional Institute of Meteorology) and Ministério da Agricultura Pecuária e Abastecimento – MAPA (Ministry of Agriculture, Livestock and Supply) (Brasil, 2020). The annual estimated population (for the middle period - July 1th) per state, from 2007 to 2019, was obtained from Instituto Brasileiro de Geografia e Estatística – IBGE (Brazilian Institute of Geography and Statistic) (IBGE, 2020).

All data on human leptospirosis cases and deaths, precipitation and state population were screened for missing records and consolidated to proceed the analysis, using Excel® 2013 Microsoft® Office (Microsoft Corporation, USA). The missing records were requested through Sistema Eletrônico do Serviço de Informação ao Cidadão – e-SIC (Electronic Citizen Information Service System).

2.2.Incidence, mortality, and case fatality rates of human leptospirosis

The incidence, mortality and case fatality rates of human leptospirosis per year, were calculated for all Brazilian regions (North, Northeast, Midwest, Southeast and South) between 2007 and 2019 (Rothman et al., 2011). For incidence, the number of human leptospirosis cases was divided by the total estimated population and multiplied by 100,000 inhabitants. For mortality, the number of human leptospirosis deaths was divided by the total estimated population and multiplied by 100,000 inhabitants. For the case fatality rate (CFR), the number of human leptospirosis deaths was divided by the number of human leptospirosis cases and multiplied by 100 inhabitants.

2.3.Time series analysis

The human leptospirosis cases were organized by monthly incidence into a time series graphic, allowing to investigate trend, seasonality and behaviors through thirteen years (2007 to 2019). Thereafter, the series was characterized as seasonal by Fisher test (Morettin and Toloi, 2006) and the model representing this series would be $Y_t = S_t + e_t$, where Y_t is the temporal series (human leptospirosis cases); S_t is the seasonality and e_t is the error. However, for the series to become stationary, seasonality was removed by a seasonality difference applied to the cases data. Then, an autocorrelation function (ACF) and an estimated partial autocorrelation function (PACF) was obtained, as an indicator of stationarity.

2.3.1. The SARIMA model

A seasonal autoregressive integrated moving average models (SARIMA) was adjusted observing the autoregressive order (*p)*, the moving average order (*q*) and the number of differences applied to the series to be stationary (d) and the *P, D, Q* that corresponds to the components of the seasonal order (Nobre et al., 2001).

A SARIMA $(p,d,q)(P,D,Q)_{12}$ with the description: $(1 - \varphi_1 B - \cdots \varphi_p B^P)(1 - \Phi_1 B^{12} - \cdots \Phi_P B^{12P})(1 - B)^d (1 - B^{12})^D Z_t = (1 - \theta_1 B - \cdots - \theta_q B_q)(1 - \Theta_1 B^{12} - \cdots \Theta_Q B^{12Q}) e_t$;

whereas $BZ_t = Z_t - 1$, $B^s Z_t = Z_{t-s}$; $(1 - \varphi_1 B - \ldots \varphi_p B^p)$, $(1 - \Phi_1 B^{12} - \ldots \Phi_P B^{12P})$ are autoregressive polynomials of p and P order respectively; $(1 - \theta_1 B - \ldots - \theta_q B_q)$, $(1 - \Theta_1 B^{12} - \ldots \Theta_Q B^{12Q})$ are moving averages polynomials of order q and Q respectively; $(1 - B)^d$ is the difference performed d times to eliminate trend; $(1 - B^{12})^D$ is the difference performed D times to eliminate seasonality; Z_t is the analyzed time series and e_t is the white noise.

An ACF and PACF to verify the adjustment of the model was performed (Nobre et al., 2001), the analysis of the residue through autocorrelation test until the order of 48 and estimation of p- value of the test allowed the verification of the adjusted model. More them one adjusted model were found for the series and their criteria of Akaike (AKAIKE, 1974), Schwarz and Hannan-Quinn (Morettin and Toloi, 2006) were compared to obtain a suitable model, adequate to the series.

The need for intervention points in the series was determined. These intervention points corresponded to points on the series in which the incidence of human leptospirosis cases was extremely high, changing the series behavior at that time. For these intervention points three intervention variables were created and a new model was generated (Table 1). The model was evaluated through the autocorrelation test of the residue, using a 48 order and estimation of p-value of the test. The criteria of this model were compared with the previous model criteria to determine which was the more suitable (Table 2).

Subsequently to determination of the most suitable model, the forecasting for new cases in the last six months of 2019 was performed and a new graphic of the series obtained. The Absolute Error Mean Percentage (AEMP) was calculated for both models of prediction, one including the interventions and another without interventions. The model with the minor AEMP

was selected as the best prediction model. Finally, a prediction model for the future cases was compared with the original data of human leptospirosis cases obtained from SINAN.

The time series analyses were performed using Gretl version 2019d software (Free Software Foundation, Italy) (Cottrell and Lucchetti, 2020).

2.4.Correlation analysis

Correlation analysis between precipitation and leptospirosis human cases was assessed using Spearman's rank correlation coefficient, after determining the non-parametric nature of the data set by the Shapiro-Wilk test (Siegel and Castellan, 1988). These analyses were performed with aid of GraphPad Prism software version 8.0 (GraphPad Software, USA).

3. Results

3.1.Incidence, mortality, and case fatality rates of human leptospirosis

The higher incidence of human leptospirosis in Brazil was in 2011 (2.60 cases / 100,000 inhabitants) and the lowest incidence was in 2017 (1.46 cases / 100,000 inhabitants), being the average annual incidence 1.913 cases / 100,000 inhabitants, between 2009 and 2017. Considering the country regions, the greater incidence, in the majority of the years assessed, was in the South region (average of 4.15 cases / 100,000 inhabitants), except for 2013, 2014 and 2015 when in the North region was observed 5.48 cases / 100,000 inhabitants, 9.97 cases / 100,000 inhabitants and 10.55 cases / 100,000 inhabitants, respectively. The lower incidence in the majority of the studied years was in the Midwest region (average of 0.44 cases / 100,000 inhabitants), except for 2015 when the Southeast region showed 0.99 cases / 100,000 inhabitants and the Midwest region 1.28 cases / 100,000 inhabitants (Fig. 1 A).

168 The mortality coefficient for human leptospirosis in Brazil (Fig.1 B) showed the great
169 value in 2011 (0.23 deaths / 100,000 inhabitants), followed by 2010 (0.20 deaths / 100,000
170 inhabitants), whereas 2016 and 2017 were the years with lowest mortality, 0.13 deaths / 100,000
171 inhabitants (both years). For mortality, between 2007 and 2019, the average was 0.168 deaths
172 / 100,000 inhabitants per year. Regarding the countries regions, the South region exhibited
173 higher values in most studied years (2007, 2008, 2010 to 2013, 2015, 2016 and 2019), with
174 greater value in 2011 (0.40 deaths / 100,000 inhabitants) and average of 0.25 deaths / 100,000
175 inhabitants per year. In 2015, North and South regions showed the same mortality rate, 0.21
176 deaths / inhabitants. However, the lowest mortality was showed in the Midwest region, in all
177 years, with average of 0.04 deaths / 100,000 inhabitants per year.
178 The results of human leptospirosis CFR in Brazil (Fig. 1 C) showed 2007 as the year with
179 highest coefficient (10.51%) among the 13 years evaluated, whereas in 2015, the CFR was the
180 lowest with 7.05%. The average annual CFR over the years evaluated was 8.82%. Analyzing
181 the lethality by region, the Southeast region showed a greater CFR in five years (2008, 2009,
182 2010, 2015 and 2019) and an average of 12.43%. Even though the Southeast showed the greater
183 CFR in the majority of years, in 2014 the Midwest region showed a CFR of 22.58%, being the
184 greater value among all studied years and regions.
185

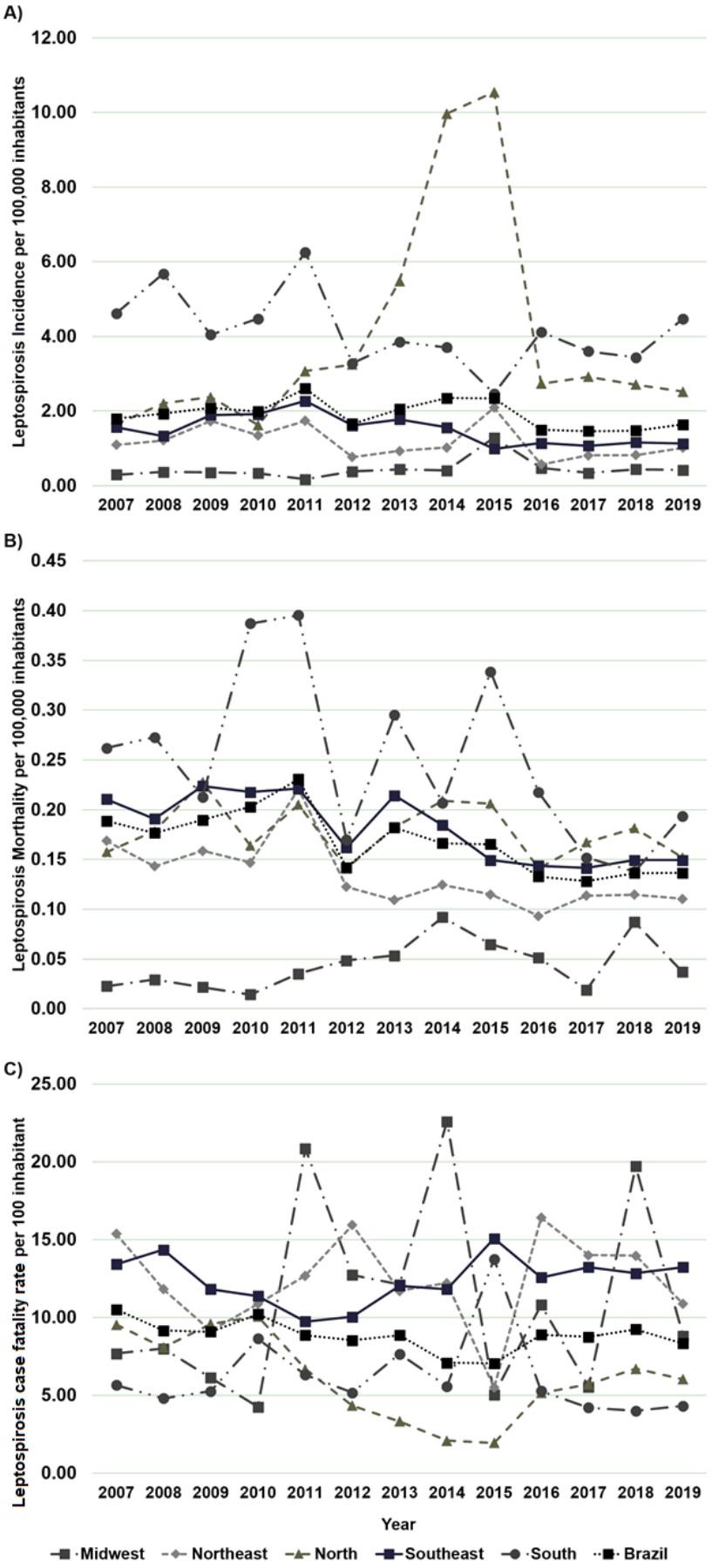
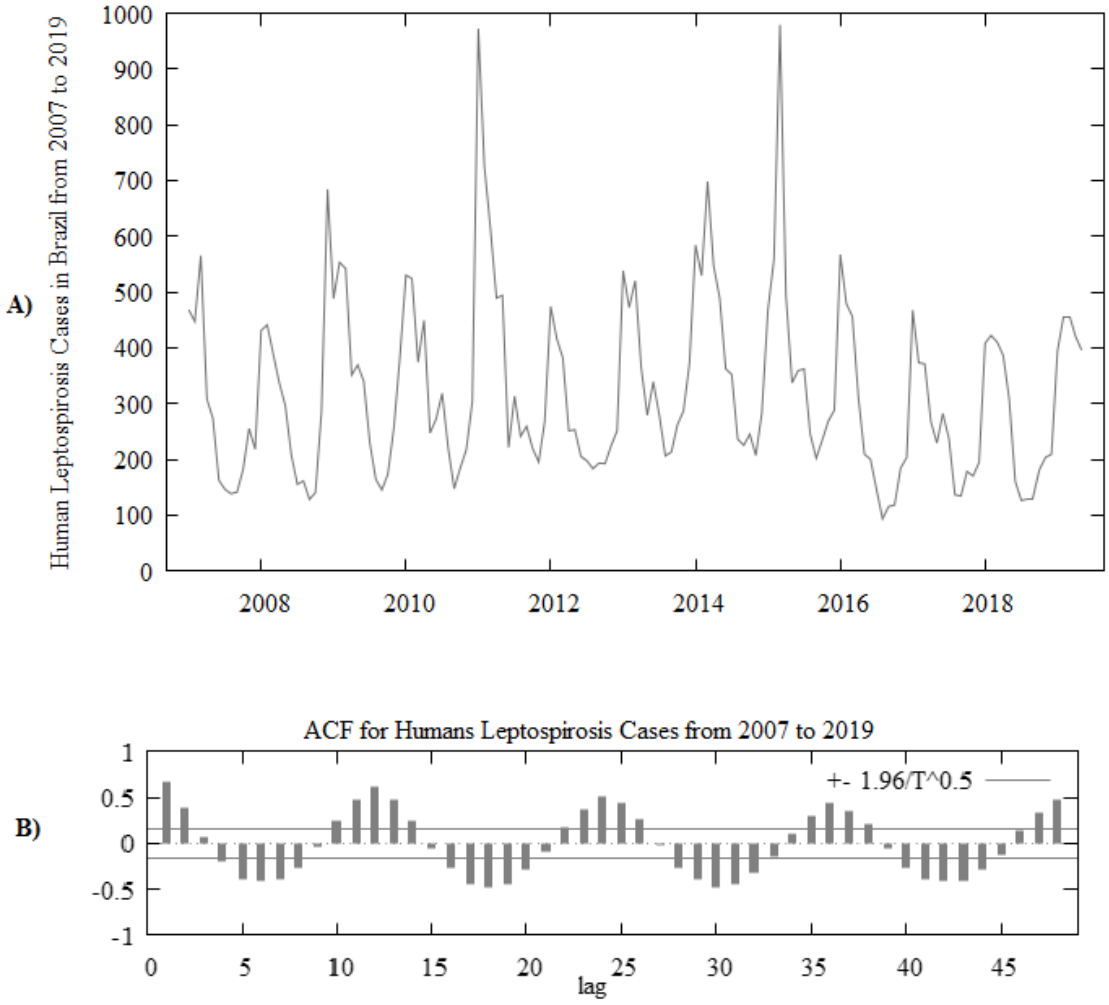

A)
Leptospirosis Incidence per 100,000 inhabitants
12.00
10.00
8.00
6.00
4.00
2.00
0.00
2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019
B)
Leptospirosis Morthality per 100,000 inhabitants
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019
C)
Leptospirosis case fatality rate per 100 inhabitant
25.00
20.00
15.00
10.00
5.00
0.00
2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019
Year
Midwest
Northeast
North
Southeast
South
Brazil

Fig.1. Indicators of human leptospirosis from January 2007 to December 2019 according to Brazilian region and for Brazil. A) Human leptospirosis incidence; B) Human leptospirosis mortality; and C) Human leptospirosis case fatality rate.

Detailed information on incidence, mortality, and CFR of human leptospirosis in Brazil according to country regions per year are shown in the supplementary material (Appendix A to C).

3.2.Temporal Series Analysis

From 2007 to 2019, the greater occurrence of human leptospirosis cases in Brazil was in 2011, with 5,009 cases and average of 200.36 cases per month. The year with fewer cases was 2017, with 2,930 cases and average of 116.89 cases per month (Fig. 2 A).



197

198 **Fig.2:** A) Distribution of human leptospirosis cases from January 2007 to December 2019 in
199 Brazil. B) Time series correlogram with 48 lags of human leptospirosis cases from January
200 2007 to December 2019 in Brazil, showing seasonality.

201 The analysis of the series graphic (Fig. 2 A) showed that the human leptospirosis cases
202 data had annual seasonality confirmed by the ACF function (Fig. 2 B). A SARIMA model built
203 using the stationary series was SARIMA $(1,0,0)(0,1,1)_{12}$. The stationary adjusted model
204 compared with the previous one was SARIMA $(0,0,1)(0,1,1)_{12}$. The first model exhibited minor
205 values in the evaluations criteria being chose to continue the analysis (Model 1: Schwarz
206 criteria: 1,658.57, Akaike criteria: 1,646.89 and Hannan-Quinn criteria: 1,651.63. Model 2:
207 Schwarz criteria: 1,668.1, Akaike criteria: 1,656.42 and Hannan-Quinn criteria: 1,661.16).

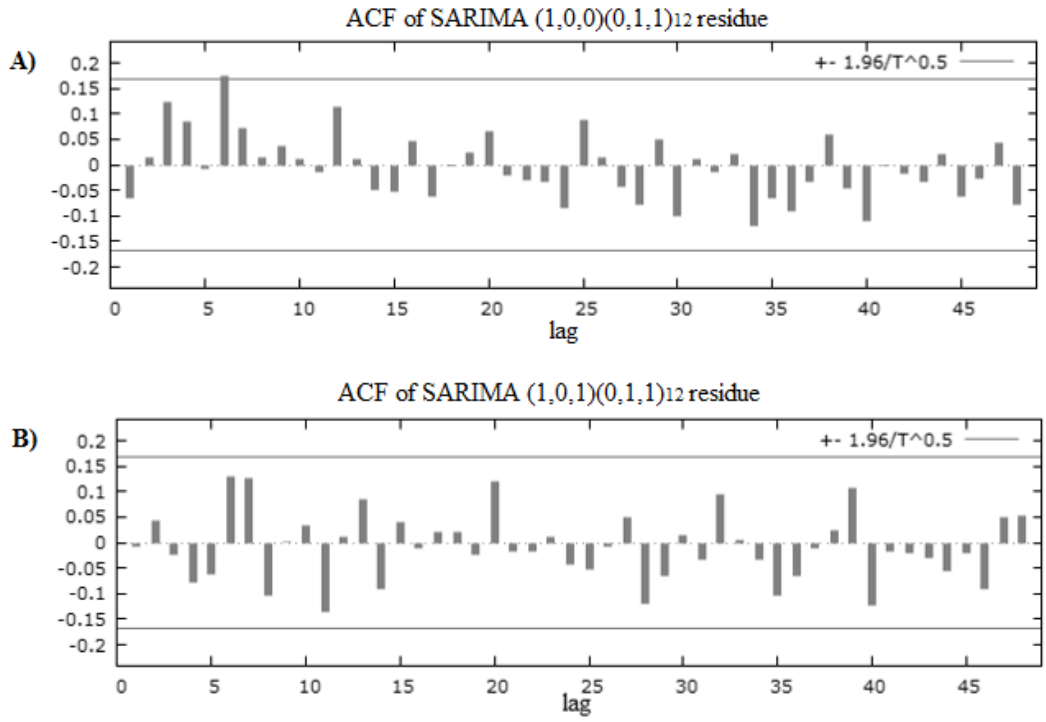
Table 1: SARIMA model SARIMA $(1,0,1)(0,1,1)_{12}$ with the ntervention selected to performed the forecast model.

Variable	Coefficient	Standard Error	p-value
Φ	0.860868	0.0640762	< 0.0001
Θ	−0.391430	0.116227	0.0008
φ_1	−0.999997	0.120088	< 0.0001
X_1	398.448	51.0011	< 0.0001
X_2	380.487	51.5834	< 0.0001
X_3	434.387	50.9796	< 0.0001

X_1 is the first intervention point, December 2008, X_2 is the second intervention point, January 2011 and X_3 is the third intervention point March 2015.

The chosen model description was $(1 - 0.49\mathrm{B})(1 - B^{12})Z_t = (1 + 1\,B^{12})e_t$. The correct adjustment of the model residue with an autocorrelation test until the order of 48 (Fig. 3 A) showed a p-value = 0.9395, which by being greater than 0.05 allowed acceptance of the null hypothesis, H_0, on what the residue was stationary.

Thereafter, the evaluation of the necessity of intervention points showed three to be intervene, these points corresponded to December 2008 (increase of 398 cases), January 2011 (increase of 380 cases) and March 2015 (increase of 434 cases). A novel model SARIMA $(1,0,1)\ (0,1,1)_{12}$ was determined considering the interventions points, being as follows: $(1 - 0.86\mathrm{B})(1 - B^{12})\mathrm{Z_t} = (1 + 0.39\mathrm{B})(1 + 0.99B^{12})\mathrm{e_t} + 398.45\mathrm{X_1} + 380.49\mathrm{X_2} + 434.39\mathrm{X_3}$ (Table 1); *X_1* is the first point of intervention – December 2008; *X_2* is the second point of intervention – January 2011; and *X_3* is the third point of intervention – March 2015. The autocorrelation test of the residue showed a p-value = 0.86, allowing the H_0 hypothesis acceptance as result of a white noise (Fig. 3 B). When compare with another model, SARIMA $(0,0,1)(0,1,1)_{12}$, the first model was more suitable due the minor values in the evaluation criteria.



226

227 **Fig. 3:** Residue autocorrelogram of the final adjusted models. A) Estimated autocorrelation
228 function (ACF) of the SARIMA $(1,0,0)(0,1,1)_{12}$ model for human leptospirosis cases from
229 January 2007 to December 2019 in Brazil after the seasonal difference, with 48 lags showing
230 that the model was correctly adjusted. B) Estimated autocorrelation function (ACF) of the
231 SARIMA $(1,0,1)(0,1,1)_{12}$ model, created with the intervention points and showed 48 lags, for
232 human leptospirosis cases from January 2007 to December 2019 in Brazil, showing the model
233 was correctly adjusted.

234 The model with interventions exhibited an AEMP value of -0.4398, whereas the model
235 without interventions showed higher AEMP value (-0.3655), therefore the selected model was
236 the one with interventions (Table 2). The predict values of new cases behaved as expected
237 (Table 3), exhibiting seasonality, with higher occurrence of cases in the rainy months (spring
238 and summer seasons) (Fig. 4 A and B).

Table 2: Forecasting models with and without the intervention points and the respectively Absolute Error Mean Percentage (AEMP) value compared with the Human leptospirosis cases from June to December 2019.

Months of 2019	With intervention		Without Intervention	
	SINAN cases	Cases Prediction	SINAN cases	Cases Prediction
June	324	303.80	324	285.64
July	193	277.33	193	258.64
August	183	210.01	183	191.76
September	167	194.70	167	176.71
October	156	212.24	156	194.58
November	168	246.15	168	228.85
December	135	286.88	135	304.56
	AEMP value:	- 0.4398	**AEMP value:**	-0.3655

SINAN = Sistema de Informação de Agravos e Notificação (Notification Disease Information System).

Table 3: Human leptospirosis cases compared to the forecast of human leptospirosis cases in Brazil from June to December 2019, considering the model with interventions SARIMA $(1,0,1)(0,1,1)_{12}$.

2019 months	SINAN cases	Cases prediction	Standard error	95% CI
June	324	303.80	54.688	196.61 - 410.99
July	193	277.33	60.41	158.92 - 395.73
August	183	210.01	64.316	83.95 - 336.06
September	167	194.70	67.059	63.27 - 326.14
October	156	212.24	69.018	76.96 - 347.51
November	168	246.15	70.432	108.10 - 384.19
December	135	286.88	71.460	146.82 - 426.94

CI = confidence interval; SINAN = Sistema de Informação de Agravos e Notificação (Notification Disease Information System).

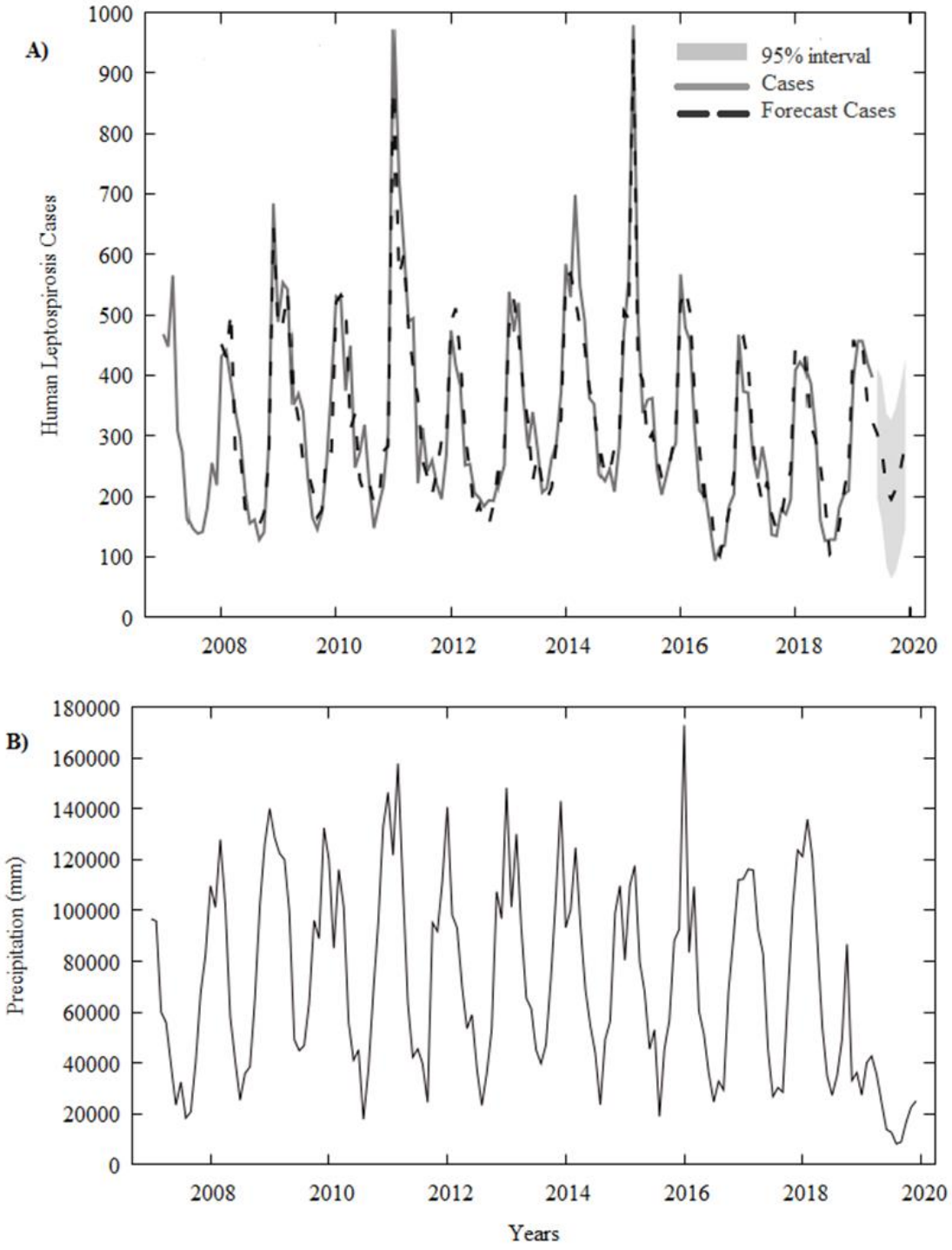


Fig.4: A) Prediction of human leptospirosis cases from June to December 2019 compared with real human leptospirosis cases in Brazil, from January 2007 to December 2019. B) Precipitation in Brazil from January 2007 to December 2019.

3.3.Correlation Analysis

Spearman coefficient for precipitation and leptospirosis human cases was 0.39 (95% confidence interval: 0.36 to 0.42, p-value < 0.001), showing a positive correlation between the variables.

4. Discussion

A temporal series analysis is a modeling tool used to describe sets of time-ordered observations, including the occurrence of diseases, being its main objectives (i) describe the series behavior and (ii) predict the series future values (Morettin and Toloi, 2006). In this context, the present study focused in develop a forecast model for human cases of leptospirosis in Brazil, considering the great impact of this disease for society (Souza et al., 2011; Torgerson et al., 2015). A model that can be very useful for the Brazilian health system to optimize resources management by strategic planning (Kretzschmar, 2020) was built.

The predicted model and the temporal series analyzed showed both annual seasonality occurrence, an expected behavior due to tropical climate of the country, with strong raining seasons in the summer (IBGE, 2010), which favor the occurrence of natural disaster as landslides and floods, well-known risk factors for human leptospirosis (Baquero and Machado, 2018). According to Word Health Organization, a tropical country usually has an incidence of human leptospirosis 10 times higher than a country of temperate climates (WHO, 2010). In fact, between 2007 and 2019 the annual average of human leptospirosis cases in Brazil was 3,791, whereas the United States normally report about 100-150 cases per year (CDC, 2020) and the European Union registered an annual average of 619.6 cases between 2011 and 2015 (ECDC, 2018). The key role of climate in the occurrence of infections by *Leptospira* spp. points toward the necessity to control the cities infrastructure to prevent landslides, floods and destructions

caused by excessive raining, which are becoming more frequent every year due to the climate change (Li et al., 2020).

Drastic climatic events preceded all the peaks of human leptospirosis observed in the temporal series analyzed (December 2008, January 2011 and March 2015) bypassed to fit the model. Regarding to these peaks, leptospirosis cases in the state of Santa Catarina were the ones that contributed most (443 cases) to the first point of intervention and were related to an intense flooding (Ghizzo Filho et al., 2018). Also, in the other points of intervention heavy rains in the state of São Paulo (284 cases only in January 2011) and Acre (509 cases) (Infoclima, 2020) were probably responsible for the atypical occurrence of the disease. This association between rain and leptospirosis cases was corroborated by the significant positive correlation found between these variables in the present study, despite the correlation coefficient was lower than expected, which can be explained due to the contribution of other risk factors to human infection in Brazil (Baquero and Machado, 2018). Indeed, the constant occurrence of leptospirosis in the dry months evidence that leptospirosis in Brazil is a health problem throughout the year, not just in the raining months, and should be always considered as differential diagnostic, through a carefully investigation, diagnostic and notification of the cases.

The analysis of the Brazilian health indicators related to leptospirosis confirm that the climate alone it is not sufficient to explain the heterogeneity of the disease incidence and mortality rates among country regions. The leptospirosis occurrence is also dependable on other aspects already identified as risk factors for the disease, such as geographical relief, agricultural production, livestock density, gross domestic product and population density (Dhewantara et al., 2020). As well as, lack of basic sanitation, poor housing state and inadequate collection of waste, conditions that usually lead to the presence of rodents near houses, increasing the probability of contact with contaminated urine and environment (Sarkar et al., 2002). In this context, the greater incidence of leptospirosis in the South region is widely attributed in the

literature to the climate, sociodemographic and agricultural characteristics (Basso and Righi, 2015; Ghizzo Filho et al., 2018; Magalhães and Acosta, 2019; Schneider et al., 2015), whereas in the North region the poor basic sanitation (Moraes et al., 2014) is probably the main risk factor for the disease occurrence, leading to exposure to the pathogen and infection. Despite the high incidence of leptospirosis in all Brazilian regions, especially compared to the rates of developed countries, the leptospirosis mortality in Brazil represented only 0.63% of the infectious and parasitic diseases mortality in the country between 2007 and 2018 (DataSUS, 2020). This low mortality rate it is possibly reflex of underestimation of the deaths caused by the disease, resulting from the misdiagnosis due to error on the differential diagnostic with other diseases, such as dengue fever, malaria or hemorrhagic fever (Benacer et al., 2016), also endemic in Brazil. An inadequate surveillance system, especially in regions of the country where the disease is not a constant concern (Baquero and Machado, 2018), the insufficient laboratory structure and inability of health professionals to distinguish and identify leptospirosis cases (Guerra, 2013), could be other reasons for underestimation of the disease incidence and mortality observed. Indeed, compared with rates observed for other developing countries with tropical climate, such as Colombia and India, which have incidence rates of 27.93 and 19.69 cases per 100,000 population and mortality rates of 1.22 and 1.12 deaths per 100,000 population, respectively (Costa et al., 2015), the incidence and mortality by leptospirosis observed in Brazil show a probable underreporting of the disease (Figure 1).

This compromised diagnostic of leptospirosis - diagnosis of severe cases only - along with the low access to health services in some parts of the country is also probably responsible for high CFR observed in Brazil between 2007 and 2019. The great ability of this indicator to point out failures in health care reveal alarming data for regions with greater CFR (Northeast, Southeast and Midwest regions). Human leptospirosis can be confused with others febrile illness in a primary diagnostic (Levett, 2001), which can lead to complications and even death,

due to late diagnostic and inappropriate treatment (Guerra, 2013; Soo et al., 2020). It is also possible that the leptospirosis deaths are result of the lack of investments in diagnostic and investigation, compared with other diseases, such as dengue fever in Brazil (Martins and Spink, 2020).

The comprehension of the social and cultural aspects of specifics communities, which affects the epidemiology of leptospirosis, must be considered for an complete control of this disease (Vijayachari et al., 2008). The gathering of reliable epidemiological data on the disease, education of health professional to recognize leptospirosis as a differential diagnostic for febrile and hemorrhagic diseases, identification of risk factors for patients and investments on diagnostic, research and environmental infrastructure are fundamental measures to control and prevent leptospirosis cases.

5. Conclusion

This study successfully developed a predict model for future cases of human leptospirosis that can be useful for the Brazilian health system to optimize the use resources and mitigate the occurrence of the disease cases. Moreover, the incidence, mortality and CFR of the disease observed highlight the need for attention and investment in the control and prevention of human leptospirosis by the Brazilian health authorities, especially in Southeast, Midwest and Northeast regions where CFR were grater.

Conflict of interests

The authors declare that there is no conflict of interest by the production of this article.

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454

455 **Appendix A:** Incidence values of all Brazilian regions of human leptospirosis cases from 2007 to 2019.

State	Region	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
DF	Midwest	1.099	0.860	1.381	1.362	0.498	0.906	1.219	0.666	1.304	1.209	0.296	0.639	0.597
GO	Midwest	0.124	0.137	0.135	0.117	0.049	0.309	0.389	0.322	0.303	0.329	0.354	0.332	0.214
MS	Midwest	0.088	0.257	0.169	0.066	0.000	0.240	0.155	0.687	0.415	0.336	0.295	0.691	0.936
MT	Midwest	0.105	0.473	0.033	0.122	0.260	0.193	0.094	0.124	3.950	0.212	0.389	0.291	0.258
AL	Northeast	1.613	2.462	2.440	2.179	2.736	1.548	1.696	2.107	1.018	0.447	2.103	1.475	1.828
BA	Northeast	0.866	0.786	1.073	1.405	1.199	0.663	1.210	0.800	0.829	0.353	0.495	0.459	0.524
CE	Northeast	0.843	1.089	3.568	0.438	1.571	0.697	0.353	0.565	0.337	0.535	0.310	0.573	1.150
MA	Northeast	0.327	0.888	0.911	0.593	0.722	0.298	0.280	0.496	1.622	0.201	0.314	0.398	0.509
PB	Northeast	0.412	0.374	0.345	0.212	0.686	0.419	0.536	0.406	3.550	0.200	0.273	0.450	0.523
PE	Northeast	2.381	2.233	2.327	3.069	4.287	1.366	1.846	2.393	1.509	1.679	2.333	2.243	2.333
PI	Northeast	0.000	0.161	0.540	0.064	0.032	0.095	0.094	0.063	15.481	0.062	0.000	0.031	0.489
RN	Northeast	0.100	0.515	1.371	0.631	1.094	0.434	0.178	0.381	2.469	0.086	0.057	0.374	0.342
SE	Northeast	4.434	3.701	2.674	3.675	2.440	1.705	1.503	2.027	0.803	0.927	1.442	1.010	1.175
AC	North	3.815	5.294	10.273	6.134	17.417	34.133	68.129	154.664	120.471	23.020	27.121	19.097	20.296
AM	North	1.521	1.377	1.916	1.091	2.120	2.005	1.628	2.117	1.930	1.125	1.747	1.593	1.158
AP	North	9.876	15.493	15.001	10.007	14.321	11.738	6.667	11.719	6.782	10.482	8.524	9.283	6.503
PA	North	1.486	1.871	1.359	1.200	1.704	1.286	1.556	1.647	1.578	1.523	1.590	1.691	1.674
RO	North	0.138	1.004	1.995	0.896	3.552	0.566	8.737	10.638	4.807	2.238	1.218	1.536	1.350
RR	North	0.505	0.727	0.474	0.444	0.000	0.639	1.024	0.805	105.208	0.000	0.383	0.347	0.330
TO	North	0.000	0.156	0.155	0.072	0.286	0.282	0.744	0.200	0.066	0.196	0.194	0.707	0.763
ES	Southeast	4.475	3.967	6.796	7.653	8.599	6.680	4.063	6.486	1.959	1.334	1.768	1.787	2.240
MG	Southeast	0.415	0.343	0.539	0.505	0.558	0.635	0.680	0.656	0.062	0.733	0.611	0.855	0.855
RJ	Southeast	1.569	1.625	2.055	1.845	2.619	1.146	1.417	1.015	0.828	0.986	0.987	1.410	1.228
SP	Southeast	1.881	1.470	2.073	2.121	2.402	1.821	2.231	1.755	1.401	1.374	1.251	1.157	1.119
PR	South	3.617	1.870	1.909	3.035	4.538	2.118	3.037	2.220	1.227	3.851	2.279	2.828	3.385

RS	South	4.734	3.952	4.224	4.451	5.068	2.545	3.914	4.283	4.845	3.571	4.363	3.972	5.994
SC	South	6.171	15.431	7.453	6.914	11.144	6.459	5.110	5.203	0.557	5.455	4.514	3.547	3.782

AC- Acre, AL – Alagoas, AM – Amazonas, AP – Amapá, BA – Bahia, CE – Ceará, DF – Distrito Federal, ES – Espírito Santo, GO – Goias, MA – Maranhão, MG – Minas Gerais, MS – Mato Grosso do Sul, MT – Mato Grosso, PA – Pará, PB – Paraíba, PE – Pernambuco, PI – Piauí, PR – Paraná, RJ – Rio de Janeiro, RN – Rio Grande do Norte, RO – Rondônia, RR – Rorâima, RS – Rio Grande do Sul, SC – Santa Catarina, SE – Sergipe, SP – São Paulo e TO – Tocantins.

Appendix B: Mortality values of all Brazilian regions of human leptospirosis cases from 2007 to 2019.

State	Region	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
DF	Midwest	0.041	0.078	0.115	0.039	0.115	0.113	0.143	0.210	0.206	0.067	0.066	0.202	0.066
GO	Midwest	0.035	0.034	0.000	0.000	0.000	0.049	0.062	0.061	0.015	0.045	0.000	0.087	0.000
MS	Midwest	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.076	0.000	0.000	0.037	0.073	0.108
MT	Midwest	0.000	0.000	0.000	0.000	0.065	0.032	0.000	0.062	0.092	0.091	0.000	0.000	0.029
AL	Northeast	0.198	0.128	0.190	0.096	0.223	0.284	0.212	0.211	0.060	0.060	0.326	0.241	0.180
BA	Northeast	0.128	0.138	0.178	0.207	0.142	0.078	0.153	0.086	0.132	0.059	0.039	0.068	0.040
CE	Northeast	0.061	0.059	0.152	0.083	0.094	0.035	0.023	0.068	0.022	0.123	0.033	0.132	0.186
MA	Northeast	0.082	0.222	0.079	0.015	0.150	0.074	0.000	0.044	0.072	0.058	0.086	0.043	0.042
PB	Northeast	0.110	0.134	0.053	0.027	0.158	0.157	0.077	0.051	0.025	0.025	0.075	0.075	0.025
PE	Northeast	0.306	0.160	0.182	0.250	0.508	0.202	0.163	0.248	0.235	0.213	0.306	0.242	0.199
PI	Northeast	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000
RN	Northeast	0.066	0.064	0.032	0.095	0.125	0.062	0.000	0.000	0.029	0.029	0.029	0.086	0.171
SE	Northeast	1.083	0.600	0.792	0.580	0.861	0.568	0.501	0.676	0.535	0.221	0.262	0.132	0.218
AC	North	0.000	0.588	1.013	0.682	0.804	0.659	0.386	0.886	0.249	0.122	0.241	0.115	0.000
AM	North	0.186	0.180	0.354	0.144	0.170	0.223	0.210	0.103	0.127	0.200	0.172	0.147	0.169
AP	North	0.000	0.163	0.160	0.000	0.438	0.286	0.000	0.799	0.000	0.383	0.251	0.844	0.591
PA	North	0.241	0.191	0.148	0.198	0.208	0.090	0.188	0.173	0.318	0.145	0.191	0.188	0.174
RO	North	0.000	0.134	0.199	0.064	0.127	0.063	0.289	0.286	0.170	0.056	0.166	0.171	0.056
RR	North	0.000	0.000	0.237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TO	North	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ES	Southeast	0.179	0.203	0.201	0.057	0.536	0.447	0.208	0.206	0.204	0.025	0.075	0.025	0.025
MG	Southeast	0.057	0.060	0.075	0.071	0.061	0.081	0.097	0.063	0.038	0.105	0.043	0.038	0.090
RJ	Southeast	0.285	0.334	0.375	0.388	0.211	0.191	0.263	0.255	0.139	0.186	0.179	0.210	0.220
SP	Southeast	0.259	0.198	0.239	0.235	0.274	0.165	0.252	0.213	0.200	0.156	0.180	0.189	0.161
PR	South	0.272	0.170	0.206	0.565	0.580	0.199	0.455	0.235	0.439	0.320	0.106	0.150	0.201
RS	South	0.302	0.304	0.174	0.234	0.270	0.158	0.233	0.214	0.293	0.159	0.185	0.168	0.229
SC	South	0.170	0.397	0.294	0.352	0.301	0.141	0.136	0.149	0.249	0.145	0.171	0.071	0.126

AC- Acre, AL – Alagoas, AM – Amazonas, AP – Amapá, BA – Bahia, CE – Ceará, DF – Distrito Federal, ES – Espírito Santo, GO – Goias, MA – Maranhão, MG – Minas Gerais, MS – Mato Grosso do Sul, MT – Mato Grosso, PA – Pará, PB – Paraíba, PE – Pernambuco, PI – Piauí, PR – Paraná, RJ – Rio de Janeiro, RN – Rio Grande do Norte, RO – Rondônia, RR – Rorâima, RS – Rio Grande do Sul, SC – Santa Catarina, SE – Sergipe, SP – São Paulo e TO – Tocantins.

Appendix C: Lethality values of all Brazilian regions of human leptospirosis cases from 2007 to 2019.

State	Region	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
DF	Midwest	3.704	9.091	8.333	2.857	23.077	12.500	11.765	31.579	15.789	5.556	22.222	31.579	11.111
GO	Midwest	28.571	25.000	0.000	0.000	0.000	15.789	16.000	19.048	5.000	13.636	0.000	26.087	0.000
MS	Midwest	0.000	0.000	0.000	50.000	0.000	0.000	0.000	11.111	0.000	0.000	12.500	10.526	11.538
MT	Midwest	0.000	0.000	0.000	0.000	25.000	16.667	0.000	50.000	2.326	42.857	0.000	0.000	11.111
AL	Northeast	12.245	5.195	7.792	4.412	8.140	18.367	12.500	10.000	5.882	13.333	15.493	16.327	9.836
BA	Northeast	14.754	17.544	16.561	14.721	11.834	11.702	12.637	10.744	15.873	16.667	7.895	14.706	7.692
CE	Northeast	7.246	5.435	4.262	18.919	5.970	5.000	6.452	12.000	6.667	22.917	10.714	23.077	16.190
MA	Northeast	25.000	25.000	8.621	2.564	20.833	25.000	0.000	8.824	4.464	28.571	27.273	10.714	8.333
PB	Northeast	26.667	35.714	15.385	12.500	23.077	37.500	14.286	12.500	0.709	12.500	27.273	16.667	4.762
PE	Northeast	12.871	7.179	7.805	8.148	11.842	14.754	8.824	10.360	15.603	12.658	13.122	10.798	8.520
PI	Northeast	0.000	0.000	0.000	0.000	0.000	0.000	0.000	50.000	0.000	0.000	0.000	0.000	0.000
RN	Northeast	66.667	12.500	2.326	15.000	11.429	14.286	0.000	0.000	1.176	33.333	50.000	23.077	50.000

SE	Northeast	24.419	16.216	29.630	15.789	35.294	33.333	33.333	33.333	66.667	23.810	18.182	13.043	18.519
AC	North	0.000	11.111	9.859	11.111	4.615	1.931	0.567	0.573	0.207	0.532	0.889	0.602	0.000
AM	North	12.245	13.043	18.462	13.158	8.000	11.111	12.903	4.878	6.579	17.778	9.859	9.231	14.583
AP	North	0.000	1.053	1.064	0.000	3.061	2.439	0.000	6.818	0.000	3.659	2.941	9.091	9.091
PA	North	16.190	10.219	10.891	16.484	12.214	7.000	12.097	10.526	20.155	9.524	12.030	11.111	10.417
RO	North	0.000	13.333	10.000	7.143	3.571	11.111	3.311	2.688	3.529	2.500	13.636	11.111	4.167
RR	North	0.000	0.000	50.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TO	North	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ES	Southeast	4.000	5.109	2.954	0.743	6.230	6.695	5.128	3.175	10.390	1.887	4.225	1.408	1.111
MG	Southeast	13.750	17.647	13.889	14.141	10.909	12.698	14.286	9.559	61.538	14.286	6.977	4.444	10.497
RJ	Southeast	18.182	20.543	18.237	21.017	8.057	16.667	18.534	25.150	16.788	18.902	18.182	14.876	17.925
SP	Southeast	13.752	13.433	11.538	11.086	11.411	9.043	11.294	12.160	14.309	11.382	14.362	16.319	14.397
PR	South	7.527	9.091	10.784	18.612	12.788	9.375	14.970	10.569	35.766	8.314	4.651	5.296	5.943
RS	South	6.387	7.692	4.121	5.252	5.331	6.204	5.950	5.000	6.055	4.467	4.251	4.222	3.812
SC	South	2.762	2.570	3.947	5.093	2.699	2.190	2.655	2.857	44.737	2.653	3.797	1.992	3.321

AC- Acre, AL – Alagoas, AM – Amazonas, AP – Amapá, BA – Bahia, CE – Ceará, DF – Distrito Federal, ES – Espírito Santo, GO – Goias, MA – Maranhão, MG – Minas Gerais, MS – Mato Grosso do Sul, MT – Mato Grosso, PA – Pará, PB – Paraíba, PE – Pernambuco, PI – Piauí, PR – Paraná, RJ – Rio de Janeiro, RN – Rio Grande do Norte, RO – Rondônia, RR – Rorâima, RS – Rio Grande do Sul, SC – Santa Catarina, SE – Sergipe, SP – São Paulo e TO – Tocantins.

CHAPTER TWO: Formatted according to the submission guidelines of Zoonosis and Public Health Journal. (Preliminary version)

Canine leptospirosis in unowned dogs: a systematic review

Short running title: Prevalence of leptospirosis in unowned dogs

Summary

The aim of this systematic review was to identify articles on prevalence of leptospirosis in sheltered and stray dogs worldwide and access the methodological quality of the recovered papers. Six databases (CABI, Cochrane, Pubmed, Scielo, Scopus and Web of Science) were searched, without restriction on year or location where the studies were performed. The search recovered 476 articles and 60 were selected for analysis according to quality criteria. None of the selected articles showed a complete explanation for the sample size adopted (probabilistic sampling), leading to the impossibility of recalculation of leptospirosis prevalence for street or sheltered dog. Among the analyzed papers 43.3% (26/60) showed five of the ten quality criteria analyzed, 16.67% (10/60) three, 15% (9/60) four, 10% (6/60) six, 6.67% (4/60) eight and only 5% (3/60) showed nine of the ten criteria analyzed. The remaining papers showed two [1.67% (1/60)] and seven [1.67% (1/60)] of the ten criteria assessed. The majority of the papers were published in the Americas [45% (27/60)] and in the last sixteen years (2003 to 2019) [80% (49/60)], and most of the sampled dogs were stray dogs [65% (39/60)]. The leptospirosis diagnostic test used more frequently was Micro Agglutination Test (MAT) [78.3% (47/60)] followed by polymerase Chain Reaction (PCR) [23.3% (14/60)], whereas the most common serovar identified was Canicola [71.4% (35/49)], Icterohaemohrragiae [65.3% (32/49)], Grippotyphosa [40.8% (20/49)] and Pomona [40.8% (20/49)]. In conclusion, our results showed that *Leptospira* spp. is present in stray and sheltered dogs worldwide, but the complete comprehension of the prevalence of leptospirosis in these populations could not be achieved

493 due to the low methodologic quality of the recovered studies about leptospirosis in stray and
494 sheltered dogs.

495 **Keywords:** seroprevalence, *Leptospira*, unowned dogs, epidemiology, cross-sectional, street
496 dogs.

497 **Impacts**

498 • Most of the selected articles did not perform a probabilistic sampling, preventing a meta-
499 analysis.
500 • Anti-*Leptospira* spp. antibodies and *Leptospira* spp. DNA were found in street and
501 sheltered dogs, diagnosed mainly by MAT and PCR.
502 • The serovars most observed in sheltered and stray dogs were Canicola,
503 Icterohaemohrragiae, Grippotyphosa and Pomona.
504

1. Introduction

Humans and dogs have lived closely for millennia (Bögel et al., 1990) and the proximity and significance of their relationship evolves every day (Cabral & Savalli, 2020). In fact, dogs help in many activities besides companionship, such as hunting, herding, guarding property, military services, law enforcement, therapeutic activities, among others (Hart & Yamamoto, 2016; WSPA, 2011). However, despite this close relationship, the population of unowned dogs is a growing on the streets around the world, especially in development countries and in places where people left their homes because of conflicts (FAO, 2014).

The number of free-roaming dogs (unrestricted owned and unrestricted unowned dogs) worldwide is estimated to be 525 million dogs (75% of world dog population) (Hughes & Macdonald, 2013; WSPA, 2011). This large stray dog population need to be managed to prevent the transmission of many zoonotic diseases, as well as dogs bites and transmission of diseases to other animals (FAO, 2014), improving animal and human health. A control measure usually implemented to minimize the problems caused by stray dogs is sheltering, a common initiative in various countries worldwide, where these dogs can be euthanized, adopted or permanently stay (Smith et al., 2019). Government, private enterprise or non-governmental organizations generally administer these shelters. Nevertheless, in many countries, euthanasia is not allowed, causing shelters to be overcrowded (Smith et al., 2019), which increases disease transmission among animals, besides other health issues.

Indeed, in animal shelters, the control of infectious diseases is a major challenge that requires a multidisciplinary approach, starting with knowledge on the epidemiologic situation of a disease and the burden caused by it (Belay et al., 2017). In this context, leptospirosis, a zoonotic disease caused by *Leptospira* spp. (Mohammed et al., 2011) has been an important concern, as it affects a variety of animals, including humans and dogs (Kurilung et al., 2019).

529 Annually, 1.03 million people are infected and 58,900 die from leptospirosis worldwide (Costa
530 et al., 2015), being dogs suggested as one of the main source of transmission to humans
531 (Kurilung et al., 2019), since they can have no clinical signs of the disease despite continue to
532 shed the bacteria in the urine (Miotto et al., 2018). The growing global number of street dogs
533 and dog shelters makes the knowledge about the epidemiological situation of zoonotic diseases,
534 such as leptospirosis, in these populations, crucial for the establishment of measures to mitigate
535 the risk of infection for caretakers, future adopters and even other animals.

536 Therefore, focusing in contribute to the control and to the knowledge about leptospirosis
537 among stray and sheltered dogs, the aim of this study was to conduct a systematic review on
538 the prevalence of canine leptospirosis in these populations. A critical review on the quality of
539 the published papers on the subject was also conducted, especially regard to the methodology
540 used by the selected studies.

541 ## 2. Material and methods

542 The PRISMA guidelines statement for cross sectional studies (Preferred Reported Items for
543 Systematic Reviews and Meta-Analyses) were adopted in this review (S1 Appendix).

544 ### 2.1.Search strategy

545 The search was conducted on September 16th, 2019. Original papers on prevalence of
546 leptospirosis in sheltered and street dogs were searched in six databases (Web of Science,
547 PubMed, Scielo, Cochrane, Scopus and Cabi), without restriction on year or location where the
548 studies were performed. The search was performed based on population (canin* and dog*),
549 intervention (shelter*, kennel* and "stray dogs"), comparison (prevalenc*) and outcome
550 (leptospir*). Detailed information on the search terms is shown in the S2 Appendix. The
551 selected keywords were investigated within all the sections from papers (title, abstract and full
552 text) in all database.

After searching the databases, the articles were imported to EndNote X7.8 (Thomson Reuters, USA) and the duplicates were removed. The screening for articles was also conducted on the reference list of the reviews recovered in the primary search.

2.2.Selection strategy and inclusion / exclusion criteria

In the first stage of the selection, all articles were screened by the title by two independent reviewers (ACTRBC and RABC) according to the selection criteria. In the second stage, the selected papers were analyzed based on the abstract (ACTRBC and RABC), whereas in the third stage, the full texts were analyzed (ACTRBC and RABC). In all stages, when the two reviewers disagreed, a third one (EMSD) was responsible for the final decision.

The following aspects were considered for the articles inclusion: (i) articles on prevalence, (ii) in shelter and / or street dogs and (iii) approach on leptospirosis. Articles focusing on: (i) leptospirosis in other species, (ii) genetics, immunology, microbiology, molecular biology, diagnostic tests, therapeutics, vaccination, and (iii) in other language than English, Spanish or Portuguese were excluded. Also, files that were not original research articles (thesis, conference proceedings, abstract and book chapter) and reviews were not selected, as well as systematic review papers. Due to the low quality identified in the recovered articles, all cross-sectional papers were selected by full-text and were further analyzed for potential limitation and bias. Full inclusion and exclusion criteria were described in Appendix S3.

2.3.Quality assessment and data extraction

Two reviewers (ACTRBC and CRP) were responsible for quality evaluation of the articles selected by full-text. This evaluation followed the National Heart, Lung and Blood Institute (NHLBI) checklist using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (Gagnier et al., 2013). Data extracted from all selected articles were: first

577 author, publication year, place where the study was carried out (city / town, county, state and
578 country, when informed), year in which sampling was performed, type of population (sheltered
579 or street dogs), number of sampled and leptospirosis-positive animals (only for stray or
580 sheltered dogs), leptospirosis diagnostic technique employed and the cut-off used (when
581 applicable) (Table 1), leptospirosis vaccination status (when available), serovars identified in
582 the serological tests (when available) (Appendix S4) and the risk factors related with occurrence
583 of leptospirosis (when available).
584

585 **Table 1**: Data extracted from all the 60 articles selected by this systematic review from the exclusion/inclusion criteria, published between 1973
586 and 2019.

First author, Year	Town / city	State / Province	Country	Sty Period	Year	Pop	Sample†	Positive‡	%Positives	Diag Method	Diag Cut off	JCR
Adesiyun, 2006	NI	NI	Trinidad and Tobago	February to July	2005	Sh. Dogs	113	5	4.42	MAT	1:100 dilution or greater was considered seropositive, 1:800 considered acute infection	1.307
Baraitareanu, 2014	Galati County, Braila County and Arges County	South-Eastern Region	Romenia	NI	NI	Sh. Dogs	77	31	40.26	PCR and MAT	1:100 dilution with 50% agglutination	0
Baraitareanu, 2019	NI	NI	Romenia	NI	NI	Sh. Dogs	19	18	94.74	PCR and MAT	NI	0
Batista, 2004	Patos	Paraíba	Brazil	February to April	2003	St. dogs	130	26	20.00	MAT	1:100 dilution with 50% agglutination	0
Belitardo, 2000	UEL	Paraná	Brazil	March to September	1998 to 1999	St. dogs	289	110	38.06	MAT/DEU/Isolation	1:100 dilution with 50% agglutination	0.37
Benacer, 2017	Kuala Lumpur/Klang Valley	Selangor	Malaysia	NI	2012 to 2013	St. dogs	150	11	7.33	Isolation/PCR/MAT/ Sequencing/PFGE	NI for MAT	0.418
Benitez, 2010	UEL	Paraná	Brazil	July to September	2007	St. dogs	33	7	21.21	MAT	1:100 dilution with 50% agglutination	0.37
Blazius, 2005	Itapema	Santa Catarina	Brazil	August to May	2000 to 2005	St. dogs	590	62	10.51	MAT	1:100 dilution with 50% agglutination	1.17
Chetta, 2014	Sicily	Sicily	Italy	April to March	2009 to 2010	Sh. dogs	183	26	14.21	PCR	NA	1.36
Chou, 2014	Taichung, Changhua and Yunlin County	Central region	Taiwan	August to July	2009 to 2011	St. dogs	720	52	7.22	PCR	NA	0.318
Cruz-Ramero, 2013	Veracruz	Veracruz	Mexico	NI	NI	Sh. dogs	92	8	8.70	MAT	Equal to or greater than 1:100 dilution	0.539
Desvars, 2013	NI	NI	Reunion Island	February and August	2009	St. dogs	50	23	46.00	MAT/PCR/qPCR	1:100 cut off	2.047
Desvars, 2012	Mayotte Island	Comoros Islands	Indian Ocean Island	March and May	2007	St. dogs	8	7	87.50	MAT/qPCR/ Sequencing	1:100 cut off	2.315
Dharanesh, 2009 §	Bangalore	Karnataka	India	NI	NI	Sh. dogs	-	79	0.00	MAT	1:100 dilution with 50% agglutination	0.227
Farrington, 1982	Guaynabo, San Juan and Mayaguez	Metropolitan Region of San Juan-Caguas-Guaynabo, Metropolitan Region of Mayaguez	Puerto Rico	June to August	1980	St. dogs	116	73	62.93	MAT	1:100 cut off	0

Feng, 2015	Kaohsiung City and Pingtung County	Southeast region	Taiwan	August to July	2009 to 2011	St. dogs	720	0	0.00	PCR and Sequencing	NA	0.318
Fonzar, 2012	Maringá	Paraná	Brazil	NI	2006 to 2008	St. dogs	355	41	11.55	MAT	NI	1.498
Goh, 2019	NI	Johore and Selangor	Malaysia	5 Months	NI	Sh. dogs	193	42	21.76	MAT	1:100 dilution with 50% agglutination	2.468
Gonçalez, 2010	Avaré	São Paulo	Brazil	NI	NI	St. dogs	300	28	9.33	MAT	NI	0.242
Hafemann, 2018	Assis Chateaubriand, São Jorge do Patrocínio, Pérola, Umuarama, Marechal Cândido Rondon, Moreira Sales, and Paranavaí.	Paraná	Brazil	March and October	2015	Sh. dogs	181	30	16.57	MAT	1:100 dilution with 50% agglutination	0.37
Ivana, 2010	Bucharest	Muntenia region	Romania	NI	NI	St. dogs	103	38	36.89	MAT	1:400 dilution and microscopic field were agglutinated	0
Jimenez-Coello, 2010	Tuxtla Gutierrez	Chiapas	Mexico	NI	NI	St. dogs	224	11	4.91	MAT	NI	0
Jimenez-Coello, 2008	Merida	Yucatan	Mexico	NI	NI	St. dogs	400	140	35.00	MAT and ELISA	1:100 dilution with 50% agglutination	2.629
Jittapalapong, 2009	Bangkok	Central Region	Thailand	NI	NI	St. dogs	230	205	89.13	MAT	1:100 dilution with 50% agglutination	0.287
Jung, 2008	Seoul	Northwest region	Korea	October and December	2005 and 2006	St. dogs	80	6	7.50	MAT	1:100 dilution with 50% agglutination	2.101
Khamesipour, 2014	Isfahan and Shahrekord	Ispaão Province and Chaharmahal and Bakhtiari Province	Iran	May and December	2013	St. dogs	30	10	33.33	PCR	NA	0.643
Khor, 2016.	NI	Selangor	Malaysia	December	2014	Sh. dogs	80	3	3.75	MAT and PCR	1:80 dilution	0.418
Kumar, 2009	Delhi	National Capital Region	India	NI	NI	St. dogs	42	4	9.52	MAT and Lipl32 ELISA	1:100 dilution with 50% agglutination	0.227
Lau, 2017	NI	NI	Malaysia	NI	NI	Sh. dogs	96	3	3.13	MAT	1:80 dilution with >50% agglutination	0.426
Mamak, 2014.	Kangal	Sivas	Turkey	NI	NI	St. dogs	29	2	6.90	MAT	NI	0.213
Manić, 2014	Leskovac	Jablanica District	Serbia	NI	NI	St. dogs	50	4	8.00	MAT	1:100 dilution	0
Medina 2010	Maracay	Aragua	Venezuela	NI	NI	Sh. Dogs	30	30	100.00	MAT	Major or equal to 1:100 title	0
Meira, 2011	Ilheus	Bahia	Brazil	NI	NI	St. dogs	100	4	4.00	PCR	NA	0.031
Miotto, 2018	São Paulo, Mogi das Cruzes and USP	São Paulo	Brazil	NI	NI	St. and	123	54	43.90	PCR and MAT	1:100 dilution with 50% agglutination	2.776

Myburgh, 1993	Pretoria	Tshwane district	South Africa	NI	1989 to 1990	Sh. Dogs St. dogs	400	7	1.75	MAT	1:160 dilution or more	0.696
Ojha, 2018	Kathmandu, Bhaktapur and Lalitpur	Kathmandu Valley	Nepal	August to January	2016 to 2017	St. dogs	70	8	11.43	ELISA Test Kit (Biogal's Immunocomb Canine Antibody Test Kit)	Identify at levels of s0 to s6, which can be low, moderate or high, cut off s3.	2.307
Oliveira, 2012¶	Porto Alegre	Rio Grande do Sul	Brazil	May and February	2007 and 2009	Sh. dogs	65	35	53.85	MAT and PCR	1:100 dilution or more	0.215
Ortega-Pacheco, 2008	Merida	Yakatan	Mexico	NI	NI	St. dogs	350	122	34.86	MAT	NI	4.295
Paz, 2015	Belém and Castanhal	Pará	Brazil	NI	2009 to 2010	Sh. dogs	141	22	15.60	MAT	1:100 dilution with 50% agglutination	1.042
Rivera Flores, 1999	Mexico City	Federal District	Mexico	NI	NI	Sh. dogs	135	52	38.52	MAT	1:100 dilution	0.2
Roach, 2010§	NI	Provinces of Kwazulu-Natal, Eastern Cape, Western Cape and Gauteng	South Africa	NI	NI	Sh. dogs	-	-	0.00	MAT	1:100 dilution with 50% agglutination	0.696
Rodríguez, 2004	Cali	Cauca Valley	Colombia	NI	2001 to 2003	St. dogs	197	81	41.12	MAT	1:100 dilution with 50% agglutination	0.733
Ryu, 1975	Tokyo, Sakai, Nagoya, Himeji, Hiroshima, Takamatsu, Matsuyama and Naha	Honshu Island, Chūbu region, Hyōgo province, Chugoku region, Kagawa province, Ehime province and Okinawa	Japan	NI	NI	St. dogs	1,615	351	21.73	Schuffner-Mochtar's Agglutination-Lysis Test	NI	0
Scanziani, 2002	Milan	Lombardia region	Italy	NI	NI	Sh. dogs	211	71	33.65	MAT	1:100 dilution to all serovars and 1:800 for Canicola and Icterohaemohrragiae	1.255
Segovia, 2013	Campeche	Yucatan Peninsula	Mexico	NI	NI	St. dogs	142	38	26.76	MAT	1:100 dilution and presented 50% agglutination or more was title with two reason dilution	1.157
Senthil, 2013	Namakkal	Tamilnadu	India	NI	NI	St. dogs	176	143	81.25	MAT	1:40 dilution 50% agglutination	0
Siam, 1973	Cairo and Giza	Cairo	Egypt	NI	NI	St. dogs	50	6	12.00	Schuffner-Mochtar's agglutination-lysis test	NI	0
Silva, 2017	Terezina	Piaui	Brazil	July to January	2010 to 2012	Sh. dogs	425	74	17.41	MAT	1:100 dilution with 50% agglutination	0
Thakur, 2014§	NI	Kathmandu	Nepal	NI	NI	St. dogs	31	-	0.00	Ig by Rapid Test Kit Method (SD Bioline).	Not apply	0

Thiermann, 1980	Detroit	Michigan	United States of America	NI	NI	St. dogs	433	164	37.88	MAT	NI	1.07
Tuemmers, 2013	Temuco	Cautín province	Mexico	18 Months	2011	St. dogs	400	85	21.25	ELISA Test Kit (Biogal's Immunocomb Canine Antibody Test Kit)	Identify at levels of s0 to s6, which can be low, moderate or high, cut off s3.	0.428
Vicari, 2007.	Palermo and Agrigento	Sicily	Italy	NI	NI	Sh. dogs	64	5	7.81	PCR	NA	0
Villanueva, 2018	Quezon City and Makati City	Manila and Lone de Taguig City-Pateros distric	Philippines	January to August	2007 to 2008	Sh. dogs	109	86	78.90	MAT and Isolation	1:80 dilution with 50% agglutination	0
Vojinović, 2015	NI	NI	Serbia	April to June	2010 to 2013	Sh. dogs	1,045	57	5.45	MAT	1:100 dilution	0.513
Weekes, 1997	NI	NI	Barbados	NI	NI	Sh. dogs	78	48	61.54	MAT	1:100 dilution	2.791
Yasuda, 1980	São Paulo	São Paulo	Brazil	October to September	1976 to 1977	St. dogs	1,415	35	2.47	Isolation	NA	0
Yasuda, 1980	São Paulo	São Paulo	Brazil	October to September.	1976 to 1977	St. dogs	1,428	308	21.57	MAT	1:100 dilution	1.968
Zaidi, 2018	Algiers	Argel province	Algeria	April to November	2017	St. dogs	104	5	4.81	qPCR/ PCR/ Sequencing	NA	2.776
Ziehl-Quirós, 2017	Guadalupe Fur Seal	Isla Guadalupe	Mexico	August	2014	St. dogs	46	12	26.09	MAT	1:50 dilution with 50% agglutination	1.659
Zwijnenberg, 2008	NI	Queensland, New South Weles, Western Australia and Northern Territory	Australia	NI	2004	Sh. dogs	956	18	1.88	MAT	≥1:50 or 1:100 to serovars L. interrogans sv. Copenhageni and sv. Australis	0.887

Sty. Period: Study Period; **Pop**: Population; **Diag. Method**: Diagnostic Method. **†:** Total of street or sheltered dogs that were sampled. ‡: Total of street or sheltered dogs that were found positive. **JCR:** Journal Citation Reports (Impact factor of the journal), value of the last available year for the journal. Accessed in June 2020. UEL: Universidade Estadual de Londrina (State University of Londrina). USP: Universidade de São Paulo (University of São Paulo). Sh. Dog: Sheltered dogs. St. dogs: Street dogs. §: Does not separate samples or results for street or sheltered dogs. ¶: Considered only positives on MAT because it was not possible to differentiate if animals positive on PCR from blood

2.4.Evaluation of potential limitations and bias of the publications included

Based on the guidelines for strengthening the reporting of observational studies in epidemiology (STROBE) (Vandenbroucke et al., 2007) and on representative samples requirements for a cross-sectional study design defined by Thrusfield (2007), ten criteria were used to assess potential limitations and bias in the articles selected by full-text, according to their presence or absence: - basic epidemiological requirements - 1) objective clearly stated; 2) location where the study (city or state or country) was carried out; 3) period when the study was carried out; 4) a clear definition of the studied population (stray or sheltered dogs); 5) a clear case definition (leptospirosis-positive); - regarding the sampling - 6) a referenced or 50% prevalence was used; 7) a level of confidence was adopted; 8) the size of sampled dog population was estimated or an infinite population was considered; 9) a statistic error was adopted; 10) the sampling performed was randomized or all animals in the population were sampled (census). For each of the quality criteria adopted, a value 1 was assigned when it was present and 0 when it was absent. At the end of the quality analysis, each study received a score according to the sum of the individual scores obtained in each criterion evaluated, which ranged from 0 to 10. Moreover, the last available impact factor of the journals where the selected papers were published were also extracted from the Journal Citation Reports (JCR) database, accessed in June 2020 (https://clarivate.com/webofsciencegroup/solutions/journal-citation-reports/).

2.5.Statistical analysis

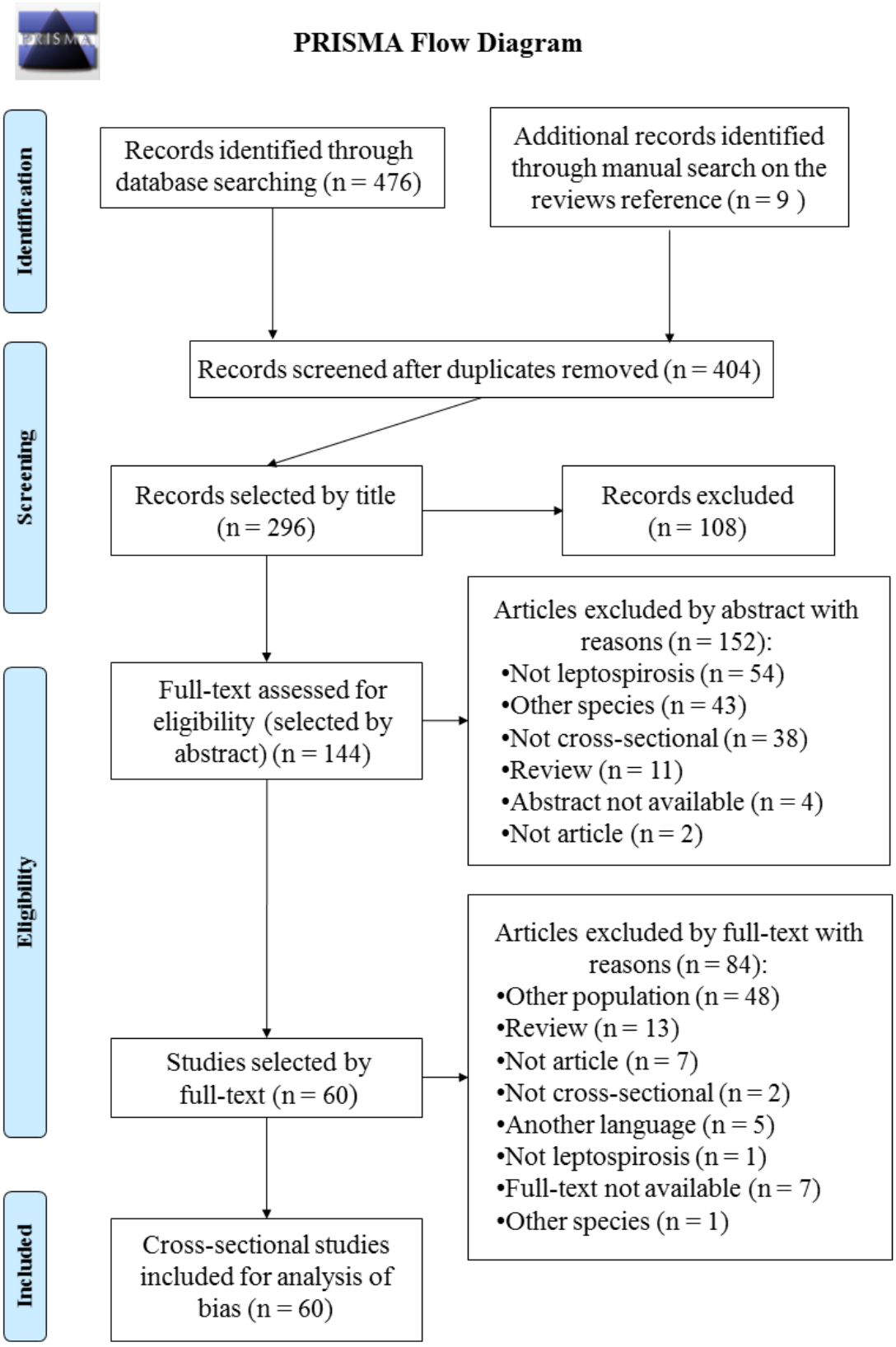
A descriptive analysis was performed on the data extracted from the selected articles. Categorical variables were analyzed by calculating proportions, while the numeric ones were analyzed by calculating the quartiles, average, median and standard deviation, when appropriated. The sampled dogs and the dogs found positives were separated by population

614 (stray and sheltered dogs), and a weighted average was calculated according to the sample size
615 for all selected articles.

616 ## 3. Results

617 ### 3.1.Main characteristics of studies included in this systematic review

618 The databases search recovered 476 articles and nine were identified by active search, 404
619 remained after duplicates (n = 81) removing and all were published between 1973 and 2019.
620 Title selection excluded 108 articles remaining 296, from these articles 144 were selected by
621 abstract, and 152 were excluded. The full-text evaluation selected 60 cross-sectional articles for
622 analysis of bias, whereas 84 were excluded (Fig. 1).



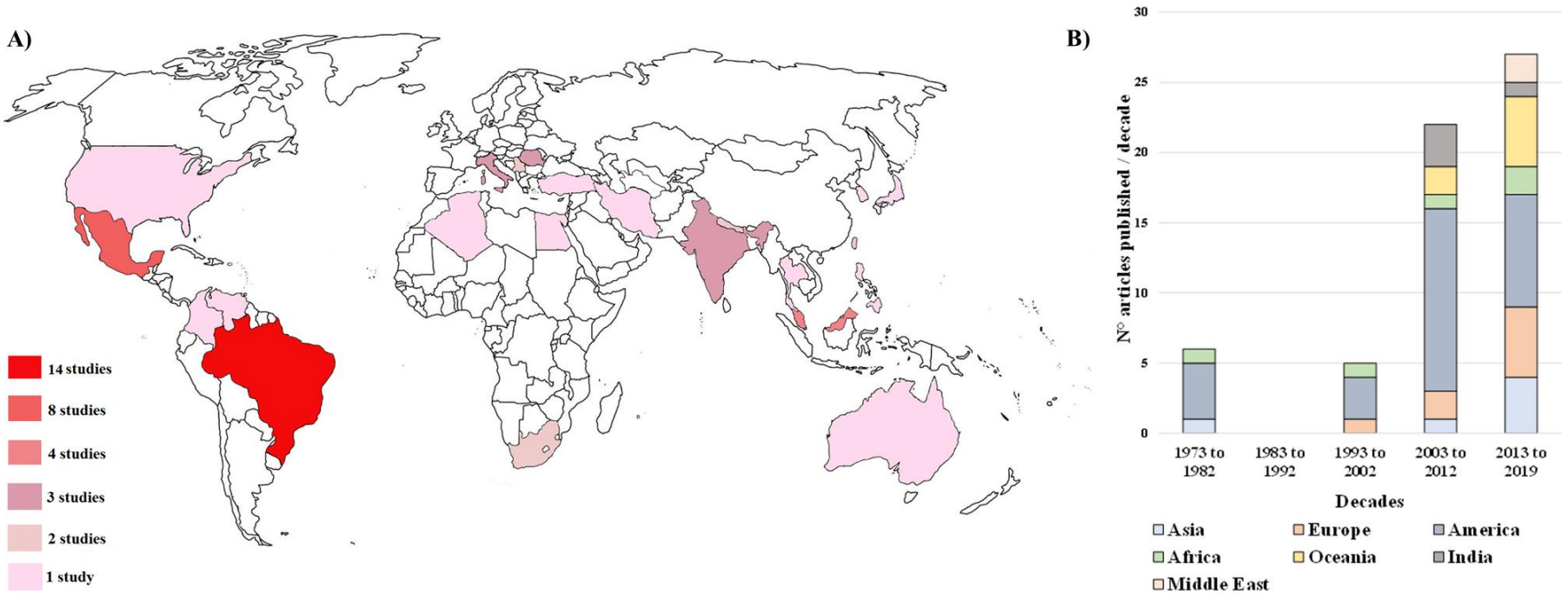
623

624 Fig.1: Flow Diagram of the articles recovered from the databases searched.

625 The majority of the selected studies was conducted in Brazil [23.4% (14/60)], followed
626 by Mexico [13.4% (8/60)], Malaysia [6.7% (4/60)], India [5% (3/60)], Italy [5% (3/60)] and
627 Romania [5% (3/60)]. Taiwan, South Africa, Serbia and Nepal represented 3% (2/60) of the
628 selected articles each. The countries with only one (1.7%) paper were Algeria, Australia,
629 Barbados, Colombia, Egypt, Indian Ocean Island (Mayotte Island), Iran, Japan, Korea,
630 Philippines, Puerto Rico, Reunion Island, Thailand, Trinidad and Tobago, Turkey, United
631 States of America and Venezuela (Fig. 2A).

632 The distribution of the year when the papers were published showed that 45% (27/60)
633 were published between 2012 and 2019, 36.7% (22/60) between 2013 and 2019, 10% (6/60)
634 between 1973 and 1982, 8.3% (5/60) between 1993 and 2002, and no article was published
635 between 1983 and 1992 (Fig. 2B). Geographical and temporal distribution of the articles
636 selected in the present study are shown in the Fig. 2.

637



638

639 Fig.2: Geographical and temporal distribution of the selected articles. A) Distribution of the selected articles according to the country where the
640 study was performed. B) Distribution of the selected articles according to the year of publication and to the continent where the study was
641 performed.

642 **3.2.Assessment of potential limitations and bias in the selected articles**

643 The analysis of the methodology of the articles showed that 43.3% (26/60) exhibit five of the
644 ten quality criteria analyzed, 16.67% (10/60) three, 15% (9/60) four, 10% (6/60) six, 6.67%
645 (4/60) eight and only 5% (3/60) showed nine of the ten criteria analyzed. The remaining papers
646 showed two [1.67% (1/60)] and seven [1.67% (1/60)] of ten criteria assessed (Table 2). The
647 final score of articles by methodological quality varied between 2 and 9, with an average and
648 median of 5, an interquartile range of 1 and standard deviation of 1.66.

649 All articles described the location where the study was conducted (city, town, municipality,
650 county, state, province or country), however six of them (10%) reported only the country.
651 Similarly, all articles specified whether the study population was from a shelter or from the
652 streets. For the others criteria, 10% (6/60) did not inform a clear objective, only 51.67% (31/60)
653 described the year when the research was conducted and 11.67% (7/60) did not exhibit a clear
654 case definition (Table 2).

655 For the evaluation of parameters that justify the sample size adopted, only 10% (6/60) of articles
656 exhibited all the criteria assessed (referenced or 50% prevalence, estimated dog population,
657 level of confidence and statistic error). Only one article (Tuemmers et al., 2013) (1.67%) used
658 50% prevalence, a level of confidence and a formula to estimate the sample size for determine
659 the prevalence for an infinite population, however did not show an error value for the
660 calculation of the sample size. Interestingly, none of the sixty selected articles specified the
661 methodology used to randomize sample collection, neither in the eight articles that used the
662 word “random”.

663 Sixteen (26.67%) of the papers were published in journals without JCR and among all the
664 journals that had JCR, the average impact factor found was 0.8296, with maximum JCR of
665 4.295 and minimum of 0.031.

666 Table 2: Evaluation of potential limitations and bias in the methodology of the 60 papers selected in this systematic review.

First author, year	Objective	Local	Period	Pop	Case def	Ref or 50% prev	Lvl of conf	Est dog pop	Stc error	Rand sample	Sum
Zwijnenberg, 2008	1	1	1	1	1	1	1	1	1	0	9
Batista, 2004	1	1	1	1	1	1	1	1	1	0	9
Ziehl-Quirós, 2017	1	1	1	1	1	1	1	1	1	0	9
Jimenez-Coello, 2008	1	1	0	1	1	1	1	1	1	0	8
Meira, 2011	1	1	0	1	1	1	1	1	1	0	8
Ojha, 2018	1	1	1	1	1	0	1	1	1	0	8
Segovia, 2013	1	1	0	1	1	1	1	1	1	0	8
Tuemmers, 2013	1	1	1	1	1	1	1	0	0	0	7
Chou, 2014	1	1	1	1	1	0	0	1	0	0	6
Paz, 2015	1	1	1	1	1	0	0	1	0	0	6
Feng, 2015	1	1	1	1	1	0	0	1	0	0	6
Fonzar, 2012	1	1	1	1	1	0	0	1	0	0	6
Khor, 2016	1	1	1	1	1	0	0	1	0	0	6
Roach, 2010	1	1	0	1	1	0	1	0	1	0	6
Adesiyun, 2006	1	1	1	1	1	0	0	0	0	0	5
Baraitareanu, 2014	1	1	0	1	1	0	0	1	0	0	5
Baraitareanu, 2019	1	1	0	1	1	0	0	1	0	0	5
Belitardo, 2000	1	1	1	1	1	0	0	0	0	0	5
Benacer, 2017	1	1	1	1	1	0	0	0	0	0	5
Benitez, 2010	1	1	1	1	1	0	0	0	0	0	5
Blazius, 2005	1	1	1	1	1	0	0	0	0	0	5
Chetta, 2014	1	1	1	1	1	0	0	0	0	0	5
Desvars, 2013	1	1	1	1	1	0	0	0	0	0	5

Desvars, 2012	1	1	1	1	1	0	0	0	0	0	5
Farrington, 1982	1	1	1	1	1	0	0	0	0	0	5
Hafemann, 2018	1	1	1	1	1	0	0	0	0	0	5
Jung, 2008	1	1	1	1	1	0	0	0	0	0	5
Khamesipour, 2014	1	1	1	1	1	0	0	0	0	0	5
Lau, 2017	1	1	0	1	1	0	0	1	0	0	5
Medina 2010	1	1	0	1	1	0	0	1	0	0	5
Miotto, 2018	1	1	0	1	1	0	0	1	0	0	5
Oliveira, 2012	1	1	1	1	1	0	0	0	0	0	5
Rodríguez, 2004	1	1	1	1	1	0	0	0	0	0	5
Scanziani, 2002	1	1	0	1	1	0	0	1	0	0	5
Silva, 2017	1	1	1	1	1	0	0	0	0	0	5
Villanueva, 2018	1	1	1	1	1	0	0	0	0	0	5
Vojinović, 2015	1	1	1	1	1	0	0	0	0	0	5
Yasuda, 1980	1	1	1	1	1	0	0	0	0	0	5
Yasuda, 1980	1	1	1	1	1	0	0	0	0	0	5
Zaidi, 2018	1	1	1	1	1	0	0	0	0	0	5
Cruz-Ramero, 2013	1	1	0	1	1	0	0	0	0	0	4
Goh, 2019	1	1	0	1	1	0	0	0	0	0	4
Ivana, 2010	1	1	0	1	1	0	0	0	0	0	4
Jittapalapong, 2009	1	1	0	1	1	0	0	0	0	0	4
Kumar, 2009	1	1	0	1	1	0	0	0	0	0	4
Myburgh, 1993	0	1	1	1	1	0	0	0	0	0	4
Ortega-Pacheco, 2008	1	1	0	1	1	0	0	0	0	0	4
Senthil, 2013	1	1	0	1	1	0	0	0	0	0	4
Weekes, 1997	1	1	0	1	1	0	0	0	0	0	4

Dharanesh, 2009	0	1	0	1	1	0	0	0	0	0	3
Gonçalez, 2010	1	1	0	1	0	0	0	0	0	0	3
Jimenez-Coello, 2010	1	1	0	1	0	0	0	0	0	0	3
Mamak, 2014	1	1	0	1	0	0	0	0	0	0	3
Manić, 2014	0	1	0	1	1	0	0	0	0	0	3
Rivera Flores, 1999	0	1	0	1	1	0	0	0	0	0	3
Siam, 1973	1	1	0	1	0	0	0	0	0	0	3
Thakur, 2014	1	1	0	1	0	0	0	0	0	;0	3
Thiermann, 1980	1	1	0	1	0	0	0	0	0	0	3
Vicari, 2007	0	1	0	1	1	0	0	0	0	0	3
Ryu, 1975	0	1	0	1	0	0	0	0	0	0	2

667 Objective: objective clearly stated; Local: location where the study (city or state or country) was carried out; Period: period when the study was carried out; Pop: a clear definition
668 of the studied population (stray or sheltered dogs); Case def.: a clear case definition (leptospirosis-positive). Ref. or 50% prev.: a referenced or 50% prevalence was used; Lvl.
669 of conf.: a level of confidence was adopted; Est. dog pop.: the size of sampled dog population was estimated or an infinite population was considered; Stc. error: a statistic error
670 was adopted; Rand. Sample: the sampling performed was randomized or all animas in the population were sampled (census). Sum: is the sum of all information showed by
671 every article. A value 1 was assigned when the characteristic assessed was present (gray cells) and 0 when it was absent (white cells).

672

3.3.Epidemiological situation of leptospirosis among stray and shelter dogs

The range of the sample size of the analyzed cross-sectional studies varied from 8 to 1,615 street or shelter dogs (average of 280.54, median of 135, standard deviation of 363.49 and interquartile range of 273). The number of positives dogs found in the studies varied from 0 to 351 (average of 51.70, median of 30, standard deviation of 69.79 and interquartile range of 55). Similarly, the relative frequency of the total sampled dogs varied from 0.05% to 10.25% (average of 1.72%, median of 0.80%, standard deviation of 2.28% and interquartile range of 1.40%) and the relative frequency of positives varied from 0% to 11.81% of the total of dogs found positive (average of 1.72%, median of 0.98%, standard deviation of 2.32% and interquartile range of 1.83%). In three articles (Dharanesh et al., 2009; Roach et al., 2010; Thakur, 2014), it was not possible to separate the sampled population, as well as the number of positive animals considering the population of dogs of interest (street or sheltered dog), from the total number of animals surveyed, therefore, these studies were excluded of these analyzes. Likewise, only Micro Agglutination Test (MAT) positive animals were considered from one study (Oliveira et al., 2012), since it was not possible to differentiate if positive- animals in Polymerase Chain Reaction (PCR) from blood were also positive PCR from urine and MAT. The distribution of sampled and test-positive dogs according to the population (street or sheltered) are shown in Table 3.

691 Table 3: Frequency of leptospirosis positive dogs weighted by the number of animals sampled for stray, sheltered and total dog population obtained
692 from the 60 papers selected in this systematic review.

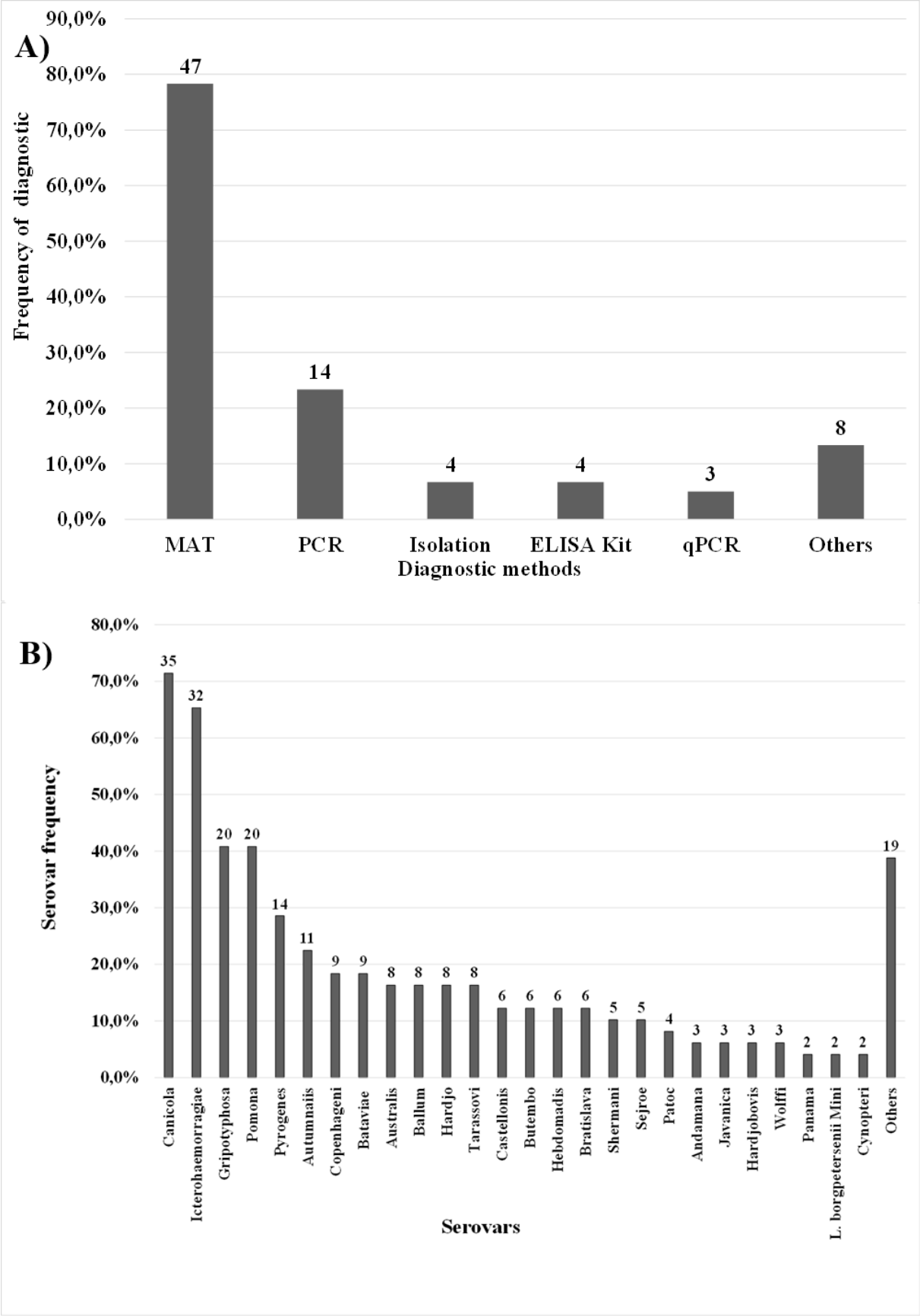
Author, year	Stray					Sheltered					Total				
	Sampled	Wt (%)	Positives	Freq (%)	Wt freq (%)	Sampled	Wt (%)	Positives	Freq (%)	Wt freq (%)	Sampled	Wt (%)	Positives	Freq (%)	Wt freq (%)
Adesiyun, 2006	NT	NT	NT	NT	NT	113	2.56	5	4.42	11.34	113	0.72	5	4.42	3.17
Baraitareanu, 2014	NT	NT	NT	NT	NT	77	1.75	31	40.26	70.31	77	0.49	31	40.26	19.68
Baraitareanu, 2019.	NT	NT	NT	NT	NT	19	0.43	18	94.74	40.83	19	0.12	18	94.74	11.43
Batista, 2004	130	1.15	26	20.00	22.92	NT	NT	NT	NT	NT	130	0.83	26	20.00	16.51
Belitardo, 2000	289	2.55	110	38.06	96.98	NT	NT	NT	NT	NT	289	1.83	110	38.06	69.83
Benacer, 2017	150	1.32	12	8.00	10.58	NT	NT	NT	NT	NT	150	0.95	12	8.00	7.62
Benitez, 2010.	33	0.29	7	21.21	6.17	NT	NT	NT	NT	NT	33	0.21	7	21.21	4.44
Blazius, 2005	590	5.20	62	10.51	54.66	NT	NT	NT	NT	NT	590	3.75	62	10.51	39.36
Chetta, 2014	NT	NT	NT	NT	NT	183	4.15	26	14.21	58.97	183	1.16	26	14.21	16.51
Chou, 2014	720	6.35	52	7.22	45.84	NT	NT	NT	NT	NT	720	4.57	52	7.22	33.01
Cruz-Ramero, 2013	NT	NT	NT	NT	NT	92	2.09	8	8.70	18.14	92	0.58	8	8.70	5.08
Desvars, 2013	50	0.44	23	46.00	20.28	NT	NT	NT	NT	NT	50	0.32	23	46.00	14.60
Desvars, 2012	8	0.07	7	87.50	6.17	NT	NT	NT	NT	NT	8	0.05	7	87.50	4.44
Farrington, 1982	116	1.02	73	62.93	64.36	NT	NT	NT	NT	NT	116	0.74	73	62.93	46.34
Feng, 2015	720	6.35	0	0.00	0.00	NT	NT	NT	NT	NT	720	4.57	0	0.00	0.00
Fonzar, 2012	355	3.13	41	11.55	36.15	NT	NT	NT	NT	NT	355	2.25	41	11.55	26.03
Goh, 2019	NT	NT	NT	NT	NT	193	4.38	42	21.76	95.26	193	1.23	42	21.76	26.66
Gonçalez, 2010	300	2.64	28	9.33	24.68	NT	NT	NT	NT	NT	300	1.90	28	9.33	17.78
Hafemann, 2018	NT	NT	NT	NT	NT	181	4.11	30	16.57	68.04	181	1.15	30	16.57	19.05
Ivana, 2010	103	0.91	38	36.89	33.50	NT	NT	NT	NT	NT	103	0.65	38	36.89	24.12
Jimenez-Coello, 2010	224	1.97	11	4.91	9.70	NT	NT	NT	NT	NT	224	1.42	11	4.91	6.98
Jimenez-Coello, 2008	400	3.53	140	35.00	123.42	NT	NT	NT	NT	NT	400	2.54	140	35.00	88.88

Jittapalapong, 2009	230	2.03	205	89.13	180.73	NT	NT	NT	NT	NT	230	1.46	205	89.13	130.14
Jung, 2008	80	0.71	6	7.50	5.29	NT	NT	NT	NT	NT	80	0.51	6	7.50	3.81
Khamesipour, 2014	30	0.26	10	33.33	8.82	NT	NT	NT	NT	NT	30	0.19	10	33.33	6.35
Khor, 2016.	NT	NT	NT	NT	NT	80	1.81	3	3.75	6.80	80	0.51	3	3.75	1.90
Kumar, 2009	42	0.37	4	9.52	3.53	NT	NT	NT	NT	NT	42	0.27	4	9.52	2.54
Lau, 2017	NT	NT	NT	NT	NT	96	2.18	3	3.13	6.80	96	0.61	3	3.13	1.90
Mamak, 2014.	29	0.26	2	6.90	1.76	NT	NT	NT	NT	NT	29	0.18	2	6.90	1.27
Manić, 2014	50	0.44	4	8.00	3.53	NT	NT	NT	NT	NT	50	0.32	4	8.00	2.54
Medina 2010	NT	NT	NT	NT	NT	30	0.68	30	100.00	68.04	30	0.19	30	100.00	19.05
Meira, 2011	100	0.88	4	4.00	3.53	NT	NT	NT	NT	NT	100	0.63	4	4.00	2.54
Miotto, 2018	7	0.06	6	85.71	5.29	116	2.63	53	45.69	120.21	123	0.78	59	47.97	37.46
Myburgh, 1993	100	0.88	7	7.00	6.17	NT	NT	NT	NT	NT	100	0.63	7	7.00	4.44
Ojha, 2018	100	0.88	8	8.00	7.05	NT	NT	NT	NT	NT	100	0.63	8	8.00	5.08
Oliveira, 2012	NT	NT	NT	NT	NT	65	1.47	53	81.54	120.21	65	0.41	53	81.54	33.65
Ortega-Pacheco, 2008	350	3.09	122	34.86	107.56	NT	NT	NT	NT	NT	350	2.22	122	34.86	77.45
Paz, 2015	NT	NT	NT	NT	NT	141	3.20	22	15.60	49.90	141	0.90	22	15.60	13.97
Rivera Flores, 1999	NT	NT	NT	NT	NT	135	3.06	52	38.52	117.94	135	0.86	52	38.52	33.01
Rodríguez, 2004	197	1.74	81	41.12	71.41	NT	NT	NT	NT	NT	197	1.25	81	41.12	51.42
Ryu, 1975	1615	14.24	351	21.73	309.44	NT	NT	NT	NT	NT	1615	10.25	351	21.73	222.83
Scanziani, 2002	NT	NT	NT	NT	NT	211	4.79	71	33.65	161.03	211	1.34	71	33.65	45.07
Segovia, 2013	142	1.25	38	26.76	33.50	NT	NT	NT	NT	NT	142	0.90	38	26.76	24.12
Senthil, 2013	176	1.55	143	81.25	126.07	NT	NT	NT	NT	NT	176	1.12	143	81.25	90.78
Siam, 1973	50	0.44	6	12.00	5.29	NT	NT	NT	NT	NT	50	0.32	6	12.00	3.81
Silva, 2017	NT	NT	NT	NT	NT	425	9.64	74	17.41	167.84	425	2.70	74	17.41	46.98
Thakur, 2014†	31	0.27	-	0.00	0.00	NT	NT	NT	NT	NT	31	0.20	0	0.00	0.00
Thiermann, 1980	433	3.82	164	37.88	144.58	NT	NT	NT	NT	NT	433	2.75	164	37.88	104.11
Tuemmers, 2013	400	3.53	85	21.25	74.94	NT	NT	NT	NT	NT	400	2.54	85	21.25	53.96
Vicari, 2007.	NT	NT	NT	NT	NT	64	1.45	5	7.81	11.34	64	0.41	5	7.81	3.17
Villanueva, 2018	NT	NT	NT	NT	NT	109	2.47	86	78.90	195.06	109	0.69	86	78.90	54.60

Vojinović, 2015	NT	NT	NT	NT	NT	1045	23.70	57	5.45	129.28	1045	6.63	57	5.45	36.19
Weekes, 1997	NT	NT	NT	NT	NT	78	1.77	48	61.54	108.87	78	0.50	48	61.54	30.47
Yasuda, 1980	1415	12.47	35	2.47	30.86		NT	NT	NT	NT	1415	8.98	35	2.47	22.22
Yasuda, 1980	1428	12.59	308	21.57	271.53		NT	NT	NT	NT	1428	9.07	308	21.57	195.53
Zaidi, 2018	104	0.92	5	4.81	4.41		NT	NT	NT	NT	104	0.66	5	4.81	3.17
Ziehl-Quirós, 2017	46	0.41	12	26.09	10.58		NT	NT	NT	NT	46	0.29	12	26.09	7.62
Zwijnenberg, 2008	NT	NT	NT	NT		956	21.68	18	1.88	40.83	956	6.07	18	1.88	11.43
Total / Weighted average (%)	11,343	100.00	2,236	26.76	51.88	4,409	100.00	735	33.12	79.38	15,752	100.00	2,971	27.62	32.52

†: Does not separate the results for street or sheltered dogs. Wt (%): Weight. Freq (%): frequency of positives dogs. Wt freq (%): Weighted frequency of positives dogs. NT: Not tested

Among all the selected papers, the most common diagnostic test used to determine the frequency of leptospirosis in dogs was MAT [78.3% (47/60)]. The second most frequent was standard PCR [23.3% (14/60)], followed by isolation and culture [6.8% (4/60)], different types of Enzyme-Linked Immunosorbent Assays (ELISA) [6.8% (4/60)] and qPCR (quantitative PCR) [5.0% (3/60)]. Other tests (Sequencing, Pulse-Field Gel Electrophoresis, Schuffner-Mochtar's agglutination-lysis test, Urine Examination in Dark Field and Rapid Test Method by SD Bioline) were used in 13.3% (8/60) of the studies (Fig.3 A). For the articles that performed MAT and Schuffner-Mochtar's agglutination-lysis test (precursor of MAT) and exhibited at least one positive animal, the most common serovar found was Canicola [71.4% (35/49)], followed by Icterohaemorrhagiae [65.3% (32/49)], Grippotyphosa [40.8% (20/49)], Pomona [40.8% (20/49)], Pyrogenes [28.6% (14/49)], Autumnalis [22.4% (11/49)] and others [38.8% (19/49)] (Fig.3 B). Among these studies, the dogs found as seropositive showed a frequency of 100% for serovars Canicola (n = 3) (Cruz-Romero, 2013; Manić et al., 2014; Medina et al., 2010), Bataviae (n = 1) (Khor et al., 2016) Hardjo (n = 1) (Medina et al., 2010) and Icterohaemohrragiae (n = 1) (Medina et al., 2010). In contrast, in one study (Blazius et al., 2005), the seropositivity for two serovars (Andamana and Wolffi) was 0.7% among the dogs found positive in MAT or Schuffner-Mochtar's agglutination-lysis test (Appendix S4).


A)
Frequency of diagnostic
90,0%
80,0%
70,0%
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
47
14
4
4
3
8
MAT
PCR
Isolation
ELISA Kit
qPCR
Others
Diagnostic methods
B)
Serovar frequency
80,0%
70,0%
60,0%
50,0%
40,0%
30,0%
20,0%
10,0%
0,0%
35
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2
19
Canicola
Icterohaemorragiae
Gripotyphosa
Pomona
Pyrogenes
Autumnaiis
Copenhageni
Bataviae
Australis
Ballum
Hardjo
Tarassovi
Castellonis
Butembo
Hebdomadis
Bratislava
Shermani
Sejroe
Patoc
Andamana
Javanica
Hardjobovis
Wolffi
Panama
L. borgpetersenii Mini
Cynopteri
Others
Serovars

713

Fig.3: A) Frequency of methods used for diagnostic of leptospirosis among the 60 articles selected by this systematic review. The group others included: Direct urine examination in a dark field (n = 1), Sequencing (n = 3), Pulsed Field Gel Electrophoresis (n = 1), Schuffner-Mochtar's agglutination-lysis test (n = 2) and Ig by rapid test kit method (SD Bioline) (n = 1). The ELISA tests used were: LipL32 ELISA (n = 1), ELISA test Kit (Biogal's Immunocomb canine antibody test kit) (n = 2) and Indirect ELISA (n = 1). B) Frequency of serovars identified by articles that performed MAT or Schuffner-Mochtar's agglutination-lysis test. Others serovars were: Ranarum, Sarmin, Louisiana, Manhao, Javanica, Manilae, Semaranga, Losbanos, Poi, Mankarso, Medanesis, Robinsoni, Arborea, Zanoni, Fort bragg, Sentot, Whiteombi, Lai and Fortbragg (one of each). MAT: Micro Agglutination test. PCR: Polymerase Chain Reaction. qPCR: Quantitative Polymerase Chain Reaction. ELISA: Enzyme Linked Immunosorbent Assay.

The vaccination status against leptospirosis among the sampled dogs was informed by 26.67% (16/60) of the studies, from which only six reported the serovars composing the vaccine and the serovars diagnosed in the sheltered and stray dogs. Among these six studies, one article (Goh et al., 2019) did not separate the serovars frequency between sheltered and owned dogs and thereby was excluded of this analysis. The comparison between the serovars exhibited by seropositive dogs and those used in the composition of the vaccines are showed in the Table 4.

Table 4: Comparison between the frequency of seropositive according to the serovars observed in Micro Agglutination Test or Schuffner-Mochtar's agglutination-lysis test and the composition of the vaccines used to vaccinated the dogs among the selected articles that informed the vaccination status of the animals sampled.

Authors, years	Pop	Serovars tested															Vaccine serovar
		Bat	Can	Pyro	Tara	Aust	Java	Icter	Gripo	Pom	Aut	Sej	Sher	Wolf	Cast	Brat	Har	
Khor, 2016	Sheltered	100%	Neg	NT	Neg	Neg	NT	Neg	Neg	Neg	NT	NT	NT	NT	NT	NT	NT	Icterohaemorrhagiae, Canicola, Pomona and Grippotyphosa
Kumar, 2009	Street	NT	50%	20%	20%	Neg	Neg	Neg	Neg	N	NT	NT	NT	NT	NT	NT	Neg	Icterohaemorrhagiae and Canicola
Lau, 2017	Sheltered	33%	Neg	Neg	Neg	33%	33%	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Icterohaemorrhagiae, Canicola, Pomona and Grippotyphosa
Miotto, 2018	Street and Sheltered	Neg	2%	26%	Neg	Neg	Neg	65%	7%	39%	83%	2%	2%	2%	Neg	Neg	Neg	Icterohaemorrhagiae, Canicola, Autumnalis and Pomona.
Scanziani, 2002	Sheltered	NT	14%	NT	1%	NT	NT	14%	35%	4%	NT	NT	NT	NT	1%	55%	3%	Icterohaemorrhagiae and Canicola

Pop: Type of population; Bat: Bataviae ; Can: Canicola; Pyro: Pyrogenes; Tara: Tarassovi; Aust: Australis; Java: Javanica; Icter: Icterohaemohrragiae; Gripo: Grippodyphosa; Pom: Pomona; Aut: Autumnailis; Sej: Serjoe; Sher: Shermani; Wolf: Wolffi; Cast: Castellonis; Brat: Bratislava; Har: Hadjo; Neg: Negative. NT: Not tested. The common serovars used in the vaccine and tested by serological tests are highlighted by grey shading.

Analysis of risk factors related to occurrence of leptospirosis was carried out by only 23.33% (14/60) of the selected articles. Of these 57.14% (8/14) did not identify any significant factor associated to leptospirosis. Among the studies that observed variables significantly associated with canine leptospirosis, the main risk factors observed were age (older than 4 years) (Chou et al., 2014) (younger than one year) (Zaidi et al., 2018), the year's season (Chou et al., 2014), the fact of being a stray dog compared with living in a household (Khamesipour et al., 2014; Paz et al., 2015; Roach et al., 2010), dogs that lived in urban areas, shared a common area with humans and exhibiting history of contact with rats (Goh et al., 2019). The detailed information on the risk factor analysis performed by these studies are found in Table 5.

Table 5: Data on the significant risk factors from the articles selected by this systematic review that performed the analysis.

First author, Year	Population	Variable	p-value	OR	95% CI
Chou, 2014†	Street dogs	*Age*	< 0.01	NI	NI
		Sampling season	< 0.001	NI	NI
Paz, 2015	Sheltered dogs	*Place*	-	-	-
		CCZ	0.04	4	1.41 to 11.0
		Shelter	Base category	-	-
		Street access	-	-	-
		Always	0.02	13.5	1.5 to 125.0
		Sometimes	Base category	-	-
Goh, 2019	Sheltered dogs	*Rat contact*‡	-	-	-
		Yes	0.043	4.61	NI
		No	Base category	-	-
		Shared common area	-	-	-
		Yes	0.002	4.51	NI
		No	Base category	-	-
Khamesipour, 2014	Street dogs	*Location*	-	-	-
		Urban	0.008	2.23	NI
		Rural	Base category	-	-
		Type Population	-	-	-
		Stray Dog	< 0.0001	NI	NI
Roach, 2010 -	Sheltered dogs	*Type Population*	-	-	-
		Stray Dogs	0.0017	NI	NI
		Province	-	-	-
		Eastern Cape	0.02	NI	NI
		Western Cape	0.02	NI	NI
Zaid, 2018	Street dogs	*Age*	-	-	-
		< 1 year	0.0001	NI	NI

OR: *Odds Ratio*. 95% CI: 95% Confidence Interval. CCZ: Centro de Controle de Zoonoses (Zoonosis Control Center). † Did not presented a base category. ‡: Adjusted *Odds ratio*. NI: Not Informed.

4. Discussion

The comprehension of a disease epidemiological situation in unowned dogs, is fundamental to implement efficient control and prevention measures (FAO, 2014), following a One Health strategy to deal with zoonosis (Mbilo et al., 2020) by understanding the disease behavior in animals, its transmission through the contaminated environment and the risk offered to humans. Therefore, the initial focus of the present systematic review was to establish the seroprevalence and risk factors of canine leptospirosis for street and sheltered dogs; however, due to low methodological quality of the papers that addressed this subject, the real situation of this important zoonosis, still remains to be determined in these animal subpopulations. Additionally, the results obtained point to the main failures performed in the selected cross-sectional studies that impaired their external validity, regarding the representativeness of the sampling, which can be used as a learning experience for the design of future studies in this field. However, it is important to mention that the determination of the leptospirosis prevalence was not the main objective of many of the studies evaluated, which certainly contributed to the low representativeness of the sampling performed. Some of the studies, although have performed cross-sectional studies, were focused in assess diagnostic tests or isolate and characterize *Leptospira* spp. strains circulating among the unowned dogs. Nonetheless, despite the low representativeness of the sampled populations, some conclusions could be drawn from the selected studies, such as the presence of canine leptospirosis among stray and sheltered dogs worldwide and the most frequently serovars observed.

In this review, stray and sheltered dogs were chosen as subject due to the risk that they offer to public and animal health regarding the transmission of diseases, considering these two different environments, streets and shelters (agglomeration, daily contact with caretaker and potential adopters). Nevertheless, the majority of the recovered articles did not perform sampling in a manner to represent significantly stray or sheltered dog populations, not following

779 basic epidemiological criteria to perform sampling (Thrusfield, 2007; Vandenbroucke et al.,
780 2007) (Table 2). The underrepresentation of sampling compromised the validity of the data
781 generated (Patino & Ferreira, 2018) and prevented a meta-analysis to recalculate the prevalence
782 of leptospirosis for these dogs. The correct method for estimating the prevalence of a disease is
783 to conduct a representative sampling of the target population (Sedgwick, 2014), which can be
784 performed considering the population as infinite (1), as finite (2) or performing a census (3)
785 (Bloch & Coutinho, 2002; Thrusfield, 2007). The criteria used to evaluate the methodology of
786 the recovered articles were those recommended for high quality cross-sectional studies
787 (Vandenbroucke et al., 2007), allowing inferences on the produced data and epidemiological
788 knowledge about a disease. For the studies involving stray dogs, the absence of a representative
789 sampling may be partly justified by the difficulty to estimate this population in most countries,
790 or it may also be due to the difficulty to find these animals that have no restrictions of
791 movement. Nonetheless, several recovered papers also failed to describe basic aspects of
792 scientific and epidemiological studies, beyond non-representative sampling, such as not state a
793 clear objective, or the locations and relevant dates for the study. This low methodology quality
794 among the selected articles probably explains the low impact factor (JCR) of the journals in
795 which these studies were published (Table 1). In fact, the exceptions to the low impact factors
796 were observed in ten articles published in journals with JCR greater than 2, which exhibited
797 between 4 and 9 of the 10 quality criteria analyzed.

798 The majority of selected articles were published in the last sixteen years and in
799 developing countries, such as Brazil, Mexico and Malaysia (Fig. 2), probably due to the increase
800 of the unowned dog population in these countries (Beck, 2000) and the importance that dogs
801 have in the maintenance of leptospirosis (Macpherson et al., 2000). Brazil was the country
802 where most of the recovered studies were conducted, presumably because of the great number
803 of dogs (52.2 million) in the country, which is the second worldwide in number of this domestic

804 animal (IBGE, 2013). The second country with the large number of recovered studies was
805 Mexico, which may be associated to the great stray dog population found in the country,
806 estimated in 16.1 million of animals (Cortez-Aguirre et al., 2018).

807 Despite the inferences about the target population being compromised as stated before,
808 the presence of canine leptospirosis among the unowned dogs (stray and sheltered) was
809 observed in the majority of studies (Table 3), evidencing the health risks associated with these
810 animal populations, especially considering the sheltered dogs due to the overpopulation, close
811 contact with caretakers and the risks for potential adopters. However, although present, the
812 frequency of the disease among the studies could not be compared directly, since in addition to
813 the non-representative sampling, the studies were also very heterogeneous and used different
814 diagnostic methods and cutoff points (Table 1). Likewise, the grouping and discussion of the
815 risk factors found in the recovered articles that were associated with canine leptospirosis among
816 unowned dogs were hampered due to the questionable and varied analysis performed among
817 the studies (Table 5). In general, the risk factors more associated with canine leptospirosis were
818 age and type of population (stray or owned dogs), probably because life on the street expose
819 the animal to more pathogens, living without welfare and sanitary care (starvation, malnutrition,
820 dehydration, vaccination, medication and deworming) (Jackman & Rowan, 2007).

821 Another important information that could be extracted from the selected articles was the
822 most common *Leptospira* serovars observed among seropositive dogs. Not surprisingly, serovar
823 Canicola was the most frequent, probably because dogs are the reservoir of this serovar, not
824 showing clinical signs of the disease when infected (Adler & de la Pena Moctezuma, 2010).
825 Indeed, no signs of acute leptospirosis was reported in the majority of the selected papers.
826 Subsequently, the following most common serovars were Icterohaemohrragiae, Grippotyphosa
827 and Pomona, already described as a concern for dogs in Europe (Ellis, 2015). Moreover, the
828 findings showed that the serovars Canicola, Icterohaemohrragiae, Grippotyphosa and Pomona

were present worldwide (Appendix S4) and should be considered for the definition of disease control, as well as in the formulation of vaccines used for dogs, in the same way as it is used in the United States for domestic dogs since 2001 (Schuller et al., 2015). Curiously, the vaccine status of the dogs sampled in the articles was showed only by five studies (Table 4) and of all vaccines used, only two articles exhibited the combination of the serovars Canicola, Icterohaemohrragiae, Grippotyphosa and Pomona. This suggests that the basic composition of leptospirosis vaccines for dogs should be reviewed according to serovars observed in the dog population (Ellis, 2010), after a carefully verification of the circulating serovars by isolation. In addition, the serovars observed in dogs without a known vaccinated status (Fig. 3), also call for attention on the importance of these four *Leptospira* serovars in the epidemiology of leptospirosis among stray and sheltered dogs.

The most common diagnostic test used to identify canine leptospirosis among the selected studies was MAT, probably because it is the golden standard method for the serological diagnostic of *Leptospira* spp. and indicates the most probable serovar that the dog had contact with (OIE, 2012). PCR was the most used method for leptospirosis prevalence determination through direct identification of the pathogen, being a molecular technique well established for this purpose (Merien et al., 1992). Although, the culture and isolation is stated as most sensitive, when perform by trained staff, for direct identification of the agent (OIE, 2012), in this review, it was the third most used method in the recovered articles, probably due to its peculiarities. Since *Leptospira* spp. is difficult to growth when in laboratory conditions, requiring specific media, temperature and long-time to growth (Adler, 2015; Mohammed et al., 2011).

5. Conclusion

In conclusion, our results point to a lack of reliable information on canine leptospirosis in street and sheltered dogs, and indicate the urgent need to conduct well-designed studies in this regard

853 to understand the epidemiological situation of the disease in these subpopulations. However,
854 despite the low methodological quality of the recovered cross-sectional studies, the findings
855 also showed that leptospirosis is present among unowned dogs, constituting an important threat
856 to human and animal health.

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865 **Conflict of Interest Statement**

866 The authors declare no conflict of interest.

867 **6. Reference**

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1178 **Appendices**

1179 **S1 Appendix**: PRISMA checklist

Section/topic	#	Checklist item	Reported on paragraph number #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Paragraph 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Paragraph 1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Paragraph 1 to 3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Paragraph 4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Paragraph 1
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Paragraph 4 to 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Paragraph 2 to 3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	S2 Appendix
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Paragraph 2 to 5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Paragraph 6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Paragraph 6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Paragraph 7

Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Paragraph 8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Paragraph 8
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Paragraph 7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Paragraph 7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Paragraph 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Paragraph 2 and 3
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Paragraph 4 to 7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Table 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not done
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Paragraph 4 to 7
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Paragraph 8 to 11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Paragraph 1 and 2
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Paragraph 2
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Paragraph 2 to 5
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Paragraph 1

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1182 **S2 Appendix**: Combination of terms used at each database investigated within all the sections
1183 from papers (title, abstract and full text) in all databases, as well as the number of articles found
1184 for the search performed on September 16th, 2019.

Database	Combination of words	Results
Web of Science	All Fields: ((canin* OR dog*) AND (leptospir*) AND (prevalenc*) AND (shelter* OR kennel* OR "stray dogs"))	26 articles
Pubmed	((((canin* OR dog*) AND (leptospir*) AND (prevalenc*) AND (shelter* OR kennel* OR "stray dogs"))	18 articles
Scielo	(canin* OR dog*) AND (leptospir*) AND (prevalenc*) AND (shelter* OR kennel* OR "stray dogs")	8 articles
Cochrane	(canin* OR dog*) AND (leptospir*) AND (prevalenc*) AND (shelter* OR kennel* OR "stray dogs")	0 articles
Scopus	(canin* OR dog*) AND (leptospir*) AND (prevalenc*) AND (shelter* OR kennel* OR "stray dogs")	363 articles
Cabi	(canin* OR dog*) AND (leptospir*) AND (prevalenc*) AND (shelter* OR kennel* OR "stray dogs")	61 articles

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1186 **S3 Appendix**: Inclusion and exclusion criteria for selection of studies in this systematic
1187 review.

Inclusion criteria	Exclusion criteria
• Papers of all countries • All years • Leptospirosis • Prevalence • Shelter dogs and / or "street dogs" • Studies written in English, Spanish and Portuguese	• Leptospirosis other species than dogs • Epidemiologic study in another species or case report or case series of leptospirosis in dogs • Genetics • Immunology • Microbiology • Performance of diagnostic tests • Therapeutics • Vaccination • Full text not available

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S4 Appendix: Frequency of dogs that reacted to the *Leptospira* spp. serovars on MAT or Agglutination Lysis test (MAT precursor) among the papers selected in this systematic review.

First Author, year	Country	Can (%)	Icter (%)	Gripo (%)	Aust (%)	Pom (%)	Ball (%)	Pyro (%)	Cast (%)	Aut (%)	Cop (%)	But (%)	Hebd (%)	Brat (%)	Bat (%)	Har (%)	Pat (%)	Tara (%)	Sej (%)	Sher (%)	Sar (%)	Loui (%)
Adesiyun, 2006	Trinidad and Tobago	Neg	40.00	Neg	Neg	Neg	20.00	Neg	NT	40.00	NT	NT	NT	NT	Neg	NT	Neg	NT	NT	NT	NT	NT
Baraitareanu, 2014	Romenia	NI†	NT	NI†	N	NI†	NI†	NT	NT	Neg	NT	NT	NT	NT	Neg	Neg	NT	NT	NT	NT	NT	NT
Baraitareanu, 2019.	Romenia	N	Neg	Neg	5.50	Neg	Neg	NT	NT	Neg	NT	NT	NT	NT	38.88	Neg	NT	Neg	Neg	NT	NT	NT
Batista, 2004	Brazil	N	2.50	10.00	2.50	17.50	NT	Neg	Neg	20.00	NT	7.50	7.50	NT	N	Neg	10.00	5.00	NT	7.50	NT	NT
Belitardo, 2000	Brazil	62.73	23.63	10.90	Neg	1.81	Neg	51.81	30.90	12.72	7.27	5.45	4.54	1.81	1.31	0.90	NT	Neg	Neg	Neg	NT	NT
Benacer, 2017	Malaysia	81.81	18.18	NT	Neg	Neg	NT	NT	NT	NT	NT	NT	Neg	Neg	Neg	Neg	NT	Neg	NT	NT	NT	NT
Benitez, 2010.	Brazil	71.50	14.50	Neg	Neg	NT	NT	28.80	14.30	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NT	Neg	NT	N	NT	NT
Blazius, 2005	Brazil	13.80	12.50	11.10	N	2.70	2.10	18.00	10.40	N	12.50	10.40	2.10	Neg	Neg	Neg	Neg	Neg	NT	1.40	NT	NT
Cruz-Ramero, 2013	Mexico	100.00	Neg	Neg	NT	Neg	Neg	Neg	NT	Neg	NT	NT	NT	Neg	NT	Neg	NT	Neg	NT	NT	NT	NT
Da Paz, 2015	Brazil	27.00	14.00	Neg	Neg	Neg	NT	Neg	Neg	Neg	11.00	Neg	Neg	Neg	Neg	Neg	20.00	Neg	NT	Neg	NT	NT
Desvars, 2013	Reunion Island	43.48	21.74	Neg	Neg	Neg	8.70	Neg	Neg	Neg	Neg	NT	Neg	NT	Neg	NT	NT	8.70	4.35	NT	NT	NT
Desvars, 2012	Indian Ocean Island	28.57	Neg	Neg	NT	Neg	14.28	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Dharanesh, 2009	India	NI‡	NI‡	NT	NT	NI‡	NT	NT	NT	NT	NT	NT	NT	NT	NT	NI‡	NT	NT	NT	NT	NT	NT
Farrington, 1982	PuertoRico	5.50	79.50	1.40	1.40	Neg	Neg	5.40	NT	Neg	NT	NT	Neg	NT	Neg	NT	NT	NT	Neg	NT	NT	NT
Fonzar, 2012	Brazil	21.90	Neg	4.90	Neg	2.40	NT	43.90	Neg	Neg	19.50	Neg	Neg	4.90	Neg	2.40	Neg	Neg	NT	Neg	NT	NT
Goh, 2019	Malaysia	NI†	NI†	NI†	NI†	Neg	NI†	Neg	NT	Neg	Neg	NT	Neg	NT	NI†	NI†	Neg	Neg	NT	NT	NT	NT
Gonçalez, 2010	Brazil	7.10	7.10	Neg	Neg	Neg	NT	Neg	NT	14.30	10.70	NT	NT	35.70	NT	7.10	NT	NT	NT	NT	NT	NT
Hafemann, 2018	Brazil	50.00	Neg	7.14	Neg	3.50	NT	Neg	Neg	Neg	NT	37.71	NT	Neg	Neg	Neg	NT	N	NT	Neg	NT	NT
Ivana, 2010	Romenia	81.57	18.43	Neg	Neg	Neg	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	NT	NT	NT	NT	NT
Jimenez-Coello, 2010	Mexico	Neg	Neg	Neg	NT	Neg	NT	73.00	NT	NT	NT	NT	NT	Neg	NT	Neg	NT	27.00	NT	NT	NT	NT
Jimenez-Coello, 2008	Mexico	65.00	11.40	Neg	NT	Neg	NT	7.90	NT	NT	NT	NT	NT	Neg	NT	Neg	NT	N	NT	NT	NT	NT
Jittapalapong, 2009	Thailand	Neg	Neg	2.00	NT	1.00	Neg	NT	NT	3.00	NT	NT	2.00	1.00	20.00	NT	8.00	7.00	6.00	4.00	3.00	1.00

Jung, 2008	Korea	Neg	16.66	Neg	Neg	Neg	Neg	Neg	NT	Neg	16.66	NT	Neg	Neg	Neg	Neg	Neg	Neg	66.66	NT	NT	NT
Khor, 2016.	Malaysia	Neg	Neg	Neg	Neg	Neg	NT	NT	NT	NT	NT	NT	Neg	NT	100.00	NT	NT	Neg	NT	NT	NT	NT
Kumar, 2009	India	50.00	Neg	Neg	Neg	Neg	Neg	20.00	NT	NT	NT	NT	Neg	NT	NT	Neg	NT	20.00	NT	NT	NT	NT
Lau, 2017	Malaysia	Neg	Neg	Neg	33.33	Neg	NT	Neg	NT	NT	NT	NT	Neg	NT	33.33	NT	NT	Neg	NT	NT	NT	NT
Mamak, 2014.	Turquia	NI‡	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Manić, 2014	Serbia	100.00	Neg	Neg	Neg	Neg	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	NT	NT	NT	Neg	NT	NT	NT
Medina 2010	Venezuela	100.00	100.00	23.33	NT	16.66	Neg	NT	NT	NT	NT	NT	10.00	NT	Neg	100.00	NT	NT	NT	NT	NT	NT
Miotto, 2018	Brazil	1.80	64.81	7.40	Neg	38.88	NT	25.92	Neg	83.33	Neg	Neg	Neg	Neg	Neg	Neg	NT	Neg	1.80	1.80	NT	NT
Myburgh, 1993	South Africa	Neg	Neg	Neg	NT	Neg	NT	28.57	NT	NT	Neg	NT	NT	NT	NT	Neg	NT	71.42	NT	NT	NT	NT
Oliveira, 2012	Brazil	NI‡	NI‡	Neg	Neg	Neg	NT	Neg	NT	Neg	NI‡	NT	Neg	Neg	NT	Neg	NT	Neg	NT	NT	NT	NT
Ortega-Pacheco, 2008	Mexico	88.50	7.40	Neg	NT	Neg	NT	Neg	NT	NT	NT	NT	NT	Neg	NT	Neg	NT	Neg	NT	NT	NT	NT
Rivera Flores, 1999	Mexico	26.92	21.15	Neg	Neg	Neg	NT	38.46	50.00	NT	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	NT	NT	NT
Roach, 2010	South Africa	NI‡	NI‡	Neg	NI‡	Neg	NT	NI‡	NT	NI‡	NI‡	NT	NT	NI‡	NI‡	Neg	NT	Neg	Neg	NT	NT	NT
Rodríguez, 2004	Colombia	38.30	55.60	45.70	NT	Neg	NT	NT	NT	NT	NT	NT	NT	Neg	NT	Neg	NT	NT	NT	NT	NT	NT
Ryu, 1975	Japan	91.96	95.46	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Scanziani, 2002	Italy	14.00	14.00	35.17	NT	4.20	NT	NT	1.40	NT	NT	NT	NT	54.87	NT	2.80	NT	1.40	NT	NT	NT	NT
Segovia, 2013	Mexico	15.78	13.15	1.38	NT	7.89	NT	N	NT	NT	NT	NT	NT	NT	5.26	2.63	NT	Neg	Neg	NT	NT	NT
Senthil, 2013	India	9.10	18.80	11.40	NT	10.20	NT	NT	NT	10.80	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Siam, 1973	Egypt	83.34	16.67	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Silva, 2017	Brazil	18.90	12.10	5.40	9.50	2.70	NT	6.80	9.50	16.20	5.40	12.10	N	N	N	N	NT	Neg	NT	1.40	NT	NT
Thiermann, 1980	United States of America	15.10	74.10	9.70	NT	17.30	NT	NT	NT	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Villanueva, 2018	Phillippines	Neg	1.10	12.79	Neg	5.81	NT	3.40	NT	13.95	12.79	NT	4.60	NT	NT	1.10	18.60	1.10	NT	NT	NT	NT
Vojinović, 2015	Serbia	43.70	48.90	31.50	Neg	35.00	NT	NT	NT	NT	NT	NT	NT	Neg	1.70	NT	NT	NT	1.70	NT	NT	NT
Weekes, 1997	Barbados	Neg	16.00	Neg	16.00	13.00	Neg	Neg	NT	45.00	NT	NT	NT	Neg	Neg	Neg	Neg	Neg	Neg	NT	NT	NT
Yasuda, 1980	Brazil	44.50	22.40	6.80	Neg	5.80	3.80	4.20	NT	4.20	NT	4.20	NT	NT	Neg	NT	NT	Neg	NT	Neg	NT	NT
Ziehl-Quirós, 2017	Mexico	43.75	43.75	Neg	NT	Neg	NT	NT	NT	NT	NT	NT	NT	12.50	NT	Neg	NT	Neg	NT	NT	NT	NT
Zwijnenberg, 2008	Australia	11.00	Neg	Neg	5.50	5.50	27.70	NT	NT	NT	28.00	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Can: Canicola; Icter: Icterohaemorragiae; Gripo: Grippotiphosa; Aust: Australis; Pom: Pomona; Ball: Ballum; Pyro: Pyrogenes; Cast: Castellonis; Aut: Autumnalis; Cop: Copenhageni; But: Butembo; Hebd: Hebdomadis; Brat: Bratislava; Bat: Bataviae; Har: Hardjo; Pat: Patoc; Tara: Tarassovi; Sej: Sejroe; Sher: Shermani; Sar: Sarmin; Loui: Louisiana. Neg: negative. NT: Not tested. NI: Not informed. NI†: found these serovars but does not informed the frequency. NI‡: found these serovars but does not separate the results between the owned and sheltered dogs.

(Part 2)

First Author, year	Country	Man (%)	Jav (%)	Mani (%)	Sema (%)	Losb (%)	Poi (%)	Mank (%)	Meda (%)	Rob (%)	Arb (%)	Zan (%)	F.bra (%)	Sent (%)	Whit (%)	Lai (%)	Rana (%)	Cyno (%)	Pana (%)	Mini (%)	Anda (%)	Hardj (%)	Hardjp (%)	Wolf (%)
Adesiyun, 2006	Trinidad and Tobago	NT	NT	NT	NT	NT	NT	40.00	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg
Baraitareanu, 2014	Romenia	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Baraitareanu, 2019.	Romenia	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg
Batista, 2004	Brazil	NT	2.50	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Ng	NT	NT	NT	2.50	NT	NT	5.00	Neg	NT	Neg
Belitardo, 2000	Brazil	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2.72	0.90	NT	NT	3.63	Ng	NT	NT	NT	NT	NT
Benacer, 2017	Malaysia	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Benitez, 2010.	Brazil	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	Neg	Neg	NT	NT	NT	NT	Neg
Blazius, 2005	Brazil	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	1.40	Neg	NT	0.70	NT	NT	0.70
Cruz-Ramero, 2013	Mexico	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT
Da Paz, 2015	Brazil	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	NT	NT	Neg	Neg	NT	Neg	NT	NT	Neg
Desvars, 2013	Reunion Island	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	13.04	Neg	NT	Neg	NT	NT
Desvars, 2012	Indian Ocean Island	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	57.14	NT	NT	NT	NT
Dharanesh, 2009	India	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Farrington, 1982	PuertoRico	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	12.30	NT	NT	Neg
Fonzar, 2012	Brazil	NT	N	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	NT	NT	Neg	Neg	NT	Neg	Neg	NT	Neg
Goh, 2019	Malaysia	NT	NI†	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NI†	NT	Neg	NT	NT	NT	‡	NT	NT

Gonçalez, 2010	Brazil	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	17.90	NT	NT	NT	NT	NT	NT
Hafemann, 2018	Brazil	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	Neg	NT	NT	Neg	Neg	NT	NT	NT	NT	Neg
Ivana, 2010	Romenia	NT	N	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg
Jimenez-Coello, 2010	Mexico	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	N	NT	NT	NT	NT	Neg
Jimenez-Coello, 2008	Mexico	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	9.30	NT	NT	NT	NT	Neg
Jittapalapong, 2009	Thailand	1.00	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	3.00	1.00	NT	NT	NT	1.00	NT	NT
Jung, 2008	Korea	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Khor, 2016.	Malaysia	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	NT	NT	NT	NT	NT
Kumar, 2009	India	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Lau, 2017	Malaysia	NT	33.33	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT
Mamak, 2014.	Turquia	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NT	NI
Manić, 2014	Serbia	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Medina 2010	Venezuela	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	16.66
Miotto, 2018	Brazil	NT	N	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	NT	NT	Neg	Neg	Neg	NT	NT	NT	1.80	
Myburgh, 1993	South Africa	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	N	NT	NT	NT	NT	
Oliveira, 2012	Brazil	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	
Ortega-Pacheco, 2008	Mexico	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	NT	NT	Neg	
Rivera Flores, 1999	Mexico	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	1.92	Neg	
Roach, 2010	South Africa	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	NT	Neg	
Rodríguez, 2004	Colombia	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	54.30	N	NT	
Ryu, 1975	Japan	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	
Scanziani, 2002	Italy	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
Segovia, 2013	Mexico	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
Senthil, 2013	India	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
Siam, 1973	Egypt	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	

Silva, 2017	Brazil	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	NT	NT	Neg	Neg	NT	NT	NT	NT	Neg
Thiermann, 1980	United States of America	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Villanueva, 2018	Phillippines	NT	NT	Neg	11.62	8.10	3.40	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Vojinović, 2015	Serbia	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Weekes, 1997	Barbados	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	Neg	NT	NT	NT	NT
Yasuda, 1980	Brazil	NT	Neg	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg	NT	NT	NT	Neg	NT	Neg	NT	NT	Neg
Ziehl-Quirós, 2017	Mexico	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Neg
Zwijnenberg, 2008	Australia	NT	5.50	NT	NT	NT	NT	NT	5.50	5.50	33.33	5.50	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Man: Manhao; Jav: Javanica; Mani: Manilae; Sema: Semaranga; Losb: Losbanos; Poi: Poi; Mank: Mankarso; Meda: Medanesis; Rob: Robinsoni; Arb: Arborea; Zan: Zanoni; F.bra: Fort bragg; Sent: Sentot; Whit: Whiteombi; Lai: Lai; Ran: Ranarum; Cyno: Cynopteri; Pan: Panama; Mini: L. borgpetersenii Mini; Anda: Andamana; Java: Javanica; Hardj: Hardjobovis; Hardjp: Hardjoprajitno and Wolf: Wolffi.. Neg: Negative. NT: Not tested. NI: Not Informed. NI†: found these serovars but does not informed the frequency. NI‡: found these serovars but does not separate the results between the owned and sheltered dogs.

CHAPTER THREE: Formatted according to the submission guidelines of International Journal of Infection Diseases.

Cross-sectional study of leptospirosis in dogs from a shelter in Minas Gerais State, Southeast region Brazil

Highlights

- All dogs were negative in PCR from urine.
- 7/322 (2.13%) dogs were seropositive on MAT, which 6/7 (85.71%) reacted to serovar Canicola, with titles of 100 to 200 and 1/7 (14.28%) reacted to serovar Autumnalis - Butembo with title of 200.
- Raised hematologic parameters and overweighed dogs were found to be associated with leptospirosis seropositivity.

Abstract

Objectives: The aim of this study was to estimate the prevalence and seroprevalence of leptospirosis in dogs from the shelter Parque Francisco de Assis, Lavras, Minas Gerais state, Brazil.

Methods: A cross-sectional study was conducted the sampling was during the dry season (June/July 2019). Blood and urine samples were collected from all dogs in the shelter. The leptospirosis was investigated using the polymerase chain reaction (PCR) targeting the gene *16S rRNA* from urine samples, and the antibodies anti-*Leptospira* spp. were searched using the micro agglutination test (MAT) from serum samples. Complete physical examination of all sampled animals was also performed, as well as a complete blood count.

Results: 329 dog were sampled, from which 213 (64.74%) were females and 116 (35.26%) males. All dogs were more than one year old. The results showed that all were negative in the PCR from urine samples. In MAT, only seven were seropositive in the first sampling, being 85.71% (6/7) reactive to serovar Canicola and 14.28% (1/7) to serovar Autumnalis-Butembo.

Conclusion: In conclusion, results showed no prevalence of *Leptospira* spp. and a low seroprevalence of anti-*Leptospira* spp. antibodies in dogs from the shelter Parque Francisco de Assis.

Keywords: Epidemiology, unowned dogs and prevalence.

1. Introduction

Leptospirosis is a disease caused by *Leptopira* spp., a spirochete Gram-negative bacteria (Picardeau, 2017) that affect most domestic and wild animals, including humans (Adler, 2015). The transmission of leptospirosis to humans and domestic animals occurs usually through contact with urine of infected hosts or contaminated environment (Schneider et al., 2015). The disease occurs in dogs with symptoms varying between mild to severer (Andre-Fontaine, 2006), with intermittent urine shedding when dogs are chronically infected (Miotto et al., 2018a).

Canine leptospirosis is a potentially zoonotic diseases considering that dogs can be a source of infection due the close living condition with humans (Jacob & Lorber, 2015), including the presence of this animals on the street. In fact, the great number of street dogs worldwide is a challenge issue for public and animal health, once these animals walk freely without supervision or sanitary care (FAO, 2014b). A control policy of street dogs in many countries, including Brazil, is sheltering, from where the dogs can be adopted or permanently stay, since in various countries euthanasia is not allowed (Smith et al., 2019).

In Brazil, the federal law (law n° 13,426) state that “surgical sterilization or another procedure that guarantees efficiency, safety and welfare to the animal” are the only alternatives to control the street dog population. Similarly, in Minas Gerais state, Brazil, euthanasia of animals as a strategy of population control is not allowed by law (law n° 21,970), making shelters commonly overpopulated. This condition (overpopulated shelters) is ideal to spread infectious diseases due the closer contact among the animals and of them with the shelter workers (Steneroden et al., 2011). Likewise, people interested in adoption of sheltered dogs are at risk to be infected by zoonotic infectious agents that these dogs could carry. Thereby, identify the occurrence of zoonotic diseases among sheltered dogs is extremely important to understand the risk that these dogs represent to other animals and to the humans in contact with them.

In this context, the aim of this study was to determinate the epidemiological situation of canine leptospirosis among dogs from a public shelter in the municipality of Lavras, Minas Gerais state, Brazil, by conducting a longitudinal (panel) study.

2. Material and methods

2.1.Study area and population

The study was conducted in the municipality of Lavras, which is located in Campo das Vertentes region, in Minas Gerais state, Brazil. Lavras is located at latitude 21° 14 '43 south and longitude 44° 59' 59 west, an altitude of 919 meters and has an area of 566.1 km². The climate was classified as temperate rainy, with dry winter and rainy summer (Dantas et al., 2007).

In the municipality there is no public shelter or Zoonosis Control Center (Centro de Controle de Zoonoses), a public unit that receive street animals and coordinate strategies to deal with zoonosis. However, a nonprofit association, named Parque Francisco de Assis, coordinated and administered by volunteers, was created to attend the needs and welfare of street dogs by

sheltering abandoned, sick and mistreated dogs. This institution (located at countryside) is used by the municipal government to perform environmental surveillance actions, specifically to deal with street dogs. The shelter has a dynamic population, sick dogs are treated and once healthy can be adopted, while dogs collected from the streets by the city hall are castrated and returned to the same place where they were found.

2.2.Study design and samples collection

A cross-sectional study was conduct and the sample collection was performed during the dry season (June/July 2019). All animals in the shelter were sampled, featuring a census study.

Before collecting samples, all dogs were identified (microchip), clinically examined and the following information were collected: sex, estimated age, vaccination status, fur size, weight, animal size (small, medium or big), presence of ectoparasites, rodent contact, disease and medication historic. Temperature, mucous, lymph nodes, score condition, hydration condition, feces aspect, urine color, presence of secretion, skin or any other lesions or alterations, behavior, response to the environment, posture and locomotion, respiratory frequency, bleeding presence and neurologic alterations were also evaluated.

After the clinical exam, whole blood, serum and urine samples were collected from all animals. Approximately 4 mL of blood was collected from the cephalic, saphenous or jugular veins into tubes with EDTA K3 (whole blood) and for serum samples. From the whole blood was performed blood count and DNA extraction. Erythrogram, leukogram and thrombogram were performed by a hematology analyzer (Prokan PE-6800 vet, China), complemented by manual evaluation of blood smear with qualitative evaluation of cells, and dosage of total proteins and fibrinogen. The whole blood was immediately (up to 4 hours after sampling) analyzed after sampling. Serum samples were centrifuged, separated in aliquots and stored at -20°C.

Urine samples were collected by cystocentesis guide by ultrasonography (SonoScape A6V, China) or using a urinary catheter in males (when possible), into a conical polypropylene tube. Serum and urine samples were maintained at -20°C until processing.

2.3.DNA extraction

Urine samples were pre-processed before the DNA extraction, as follows: 1 mL was centrifuged at 12.000 x g for 10 minutes at room temperature, the supernatant was removed and the pellet re-suspended in 200 µL phosphate buffered saline (PBS) (0.01 M, pH 7.4, all from Sigma-Aldrich, USA). DNA extraction was performed using PureLink® Genomic DNA Kit (Invitrogen™, Thermo Fisher Scientific, USA) following the manufacture's recommendations.

2.4.*Lepstospira* spp. polymerase chain reaction (PCR)

The investigation of *Leptospira* spp. DNA in the urine samples was carried out by amplification of the gene *16S rRNA* (PCR) using the primer pairs Lep1 5'-GGCGGCGCGTCTTAAACATG-3' and Lep2 5'-TTCCCCCCATTGAGCAAGATT-3' (Merien et al., 1992b). Expected amplified fragment was 330 bp. The PCR reaction was performed with a mix containing, 1X buffer (500 mM KCl, 100 mM Tris-HCl, 1% Triton X-100 and pH 8,4), 3 mM of $MgCl_2$, 0.2 mM of dNTP, 0.5 µM of each primer, 2.5 U/mL of Taq (Phoneutria Biotecnologia e Serviços Ltda – PHT, Brazil) and the DNA template. The amplification conditions were: initial denaturation of 5 minutes at 94 ºC; 40 cycles of 94 ºC for 30 seconds, 60 ºC for 30 seconds and 72 ºC for 30 seconds; and final extension of 72 ºC for 5 minutes. DNA from *L. biflexa* serovar Patoc, a strain from the collection of the Laboratório de Zoonoses Bacterianas, Universidade de São Paulo, was used as positive control and ultrapure water as negative control in all assays. The analyses of all amplicons were performed at 1.0% agarose gel electrophoresis and visualized under ultraviolet light.

2.5.Microscopic agglutination test (MAT)

Antibodies against *Leptospira* spp. were detected by microscopic agglutination test (MAT), according to Galton et al. (1965) and Cole et al. (1973), using a collection of 24 serovars, including reference strains and indigenous strains isolated in Brazil (Table 1). Sera with titers ≥ 100 were considered reactive and the antigen that presented the highest titer was considered as the infective serogroup (Adler, 2015). The final titer was the reciprocal highest dilution of the sample in which 50% or more of agglutinated *Leptospira* spp. were observed. MAT was performed at Laboratorio de Zoonoses Bacterianas, Universidade de São Paulo (University of São Paulo), São Paulo, São Paulo state, Brazil.

Table 1: Serovars of *Leptospira* spp. used in the microscopic agglutination test (MAT) for testing dogs from Parque Francisco de Assis, Lavras, Minas Gerais state, Brazil, 2019-2020.

Species	**Serogroups**	**Serovar**
	Ballum	Castellonis
	Sejroe	Hardjo (Hardjobovis)
L. borgpetersenii	Javanica	Javanica
	Tarassovi	Tarassovi
	Celledoni	Whitcombi
	Australis	Australis
	Autumnalis	Autumnalis
	Bataviae	Bataviae
	Australis	Bratislava
	Canicola	Canicola
	Icterohaemorrhagiae	Copenhageni
L. interrogans	Sejroe	Hardjo (Hardjoprajitno)
	Hebdomadis	Hebdomadis
	Pomona	Pomona
	Pomona	Pomona (GR6)
	Pyrogenes	Pyrogenes
	Icterohaemorrhagiae	Icterohaemorrhagiae
	Djasiman	Sentot
	Grippotyphosa	Grippotyphosa
L. kirschneri	Autumnalis	Butembo
	Cynopteri	Cynopteri
L. noguchi	Panama	Panama
L. santarosai	Shermani	Shermani
	Sejroe	Guaricura

2.6.Statistical analysis

Apparent prevalence (PCR) and seroprevalence (MAT) of leptospirosis were calculated by dividing the number of test-positive results by the total number of tested dogs. The 95% confidence intervals (CI) for these prevalences were obtained by the exact binomial distribution using the package “binom”(Dorai-Raj & Dorai-Raj, 2009) with aid of R software version 4.0.2 (Team, 2018)

2.7.Ethics statement

This study was approved by the ethic committee of the Universidade Federal de Lavras (Comissão de Ética no Uso de Animais – CEUA/UFLA) under the protocol number 117/2018. All animal manipulations followed international animal welfare guidelines (Ryan et al., 2019).

3. Results

3.1.Population description

In the sampling, 329 dogs were sampled, from which 213 (64.74%) were females and 116 (35.26%) males. All dogs were more than one year old, being 59% (194/329) between 5 and 7 years, 25.5% (84/329) between 8 and 10 years, 14.9% (49/329) between 2 and 4 years and only 0.6% (2/329) were more than 10 years old.

All dogs were vaccinated against leptospirosis, in September 2018. The vaccine used was INOMUNE (Ceva, France), containing the *Leptospira* serovars Canicola, Icterohaemorrhagiae, Pomona and Grippotyphosa.

3.2.Leptospirosis prevalence

All animals exhibited negative results in *Leptospira* spp. PCR performed from urine samples, [0/329, 95% CI (0 to 0.0111)].

3.3.Leptospirosis seroprevalence

The seroprevalence of leptospirosis in the shelter was 2.13% (7/322) (95% CI: 0.86 to 4.33). Among the positive animals, 6 (85.71%) were female and one (14.29%) male. The serovars found were Canicola [6/7 (85.71%)] with titers varying from 100 to 200 and Autumnalis - Butembo [1/7 (14.29%)], both reactive with the titer of 200. Detailed information on MAT results are shown in Table 2.

Table 2: Results of leptospirosis micro agglutination test (MAT) for dogs from a public shelter in the municipality of Lavras, Minas Gerais State, Brazil, sampled during the dry season 2019 (July/August).

Animal	Sex	Age	*Leptospira* serovar	Title
8254	Female	7 years	Autumnalis - Butembo	200
8321	Female	7 years	Canicola	100
8325	Male	7 years	Canicola	100
8328	Female	7 years	Canicola	100
8341	Female	7 years	Canicola	100
8392	Female	8 years	Canicola	200
8416	Female	7 years	Canicola	100

4. Discussion

The knowledge about the prevalence and incidence of a zoonotic disease, such leptospirosis, in dog shelters are fundamental to access the risk that these animals represents to other animals, but especially to humans that are in close contact with then or future adopters (Macpherson et al., 2000). In this context, the preliminary results of the present study showed a low risk associated with leptospirosis among dogs from the shelter of the municipality of Lavras, Minas Gerais, although they evidenced the presence of anti-*Leptospira* spp. antibodies among the dogs, reinforcing the importance of prevention measures against leptospirosis.

In this sense, the negative PCR results showed that no animal was shedding *Leptospira* spp. in urine and thereby did not offer a risk of infection to humans, other dogs and contamination of the environment (Khorami et al., 2009). The strict rodent control practiced monthly in the shelter, by a specialized company, is the most likely explanation for the absence of infection observed, in addition to the regular vaccination of animals and the conditions of high hygiene practiced daily in the shelter facilities. These same reasons also explain the low prevalence of seropositive animals observed in the MAT results. Despite the low frequency, the seropositive results possibly indicate previous contact with the pathogen. These findings could also reflect vaccination titles, since the vaccine used by the dog shelter contains serovar Canicola, however this is unlikely considering that titles resulting from vaccination have few months duration (Klaasen et al., 2003).

Among seropositive animals, the serovar Canicola was the most frequent, probably because it is a serovar adapted to dogs (Adler & de la Pena Moctezuma, 2010). On the other hand, one animal was reactive to serovar Autumnalis-Butembo suggesting previous contact with farm animals, such as horses, goats, sheep or cattle (Ellis, 2015; Krijger et al., 2019; Oliveira, S. V. d. et al., 2012b; Silva, F. J. d. et al., 2015), contact with rodents (Krijger *et al.*, 2019) or even with other dogs (Jorge et al., 2017). In this context, it is important to mention that the shelter is located in a rural area, favoring the contact with livestock and wild animals. Nonetheless, it is also important to note that, since the animals come from different origins, they could have had contact with *Leptospira* spp. before to be in the shelter.

Overall, the low leptospirosis seroprevalence observed for the dogs shelter strengthens the importance of adopting rodent control measures, vaccination and hygiene, to protect humans, animals and the environment against leptospirosis, showing a clear application of the One Health concept to deal with zoonosis.

5. Conclusion

In conclusion, the results showed a low prevalence of anti-*Leptospira* spp. antibodies in dogs from the shelter Parque Francisco de Assis.

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2. GENERAL CONCLUSIONS

In conclusion, this dissertation demonstrated, in the first chapter, a predict model for future cases of human leptospirosis and the rates of incidence, mortality and lethality of the disease, that highlight the need for attention and investment in the control and prevention of human leptospirosis in Brazil. Additionally, in the second chapter, the results pointed to a lack of reliable information on the prevalence of canine leptospirosis in street and sheltered dogs, however, the findings also showed that leptospirosis is present among unowned dogs worldwide, constituting an important threat to human and animal health. Finally, the third chapter results, from the cross-sectional study, showed a low prevalence of anti-*Leptospira* spp. antibodies in dogs from the shelter Parque Francisco de Assis located in the municipality of Lavras, Minas Gerais state, Brazil.

3. FINAL CONSIDERATIONS

The production of knowledge and science by universities and research institutions should substantiate public policies and encourage the evolution of the available information in the literature. Therefore, the studies composing this dissertation are potential tools that can help the public health agencies to deal with leptospirosis in humans and with risks associated with unowned dogs. However, the lack of representative available data about the epidemiological situation of canine leptospirosis in unowned dogs, represent a great challenge for human and animal health and an obstacle to the correct implementation of control and prevention measures for the disease.