Canine leptospirosis in stray and sheltered dogs: a systematic review

Short running title: Prevalence of leptospirosis in unowned dogs

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Summary

The aim of this systematic review was to identify articles on prevalence of leptospirosis in stray and sheltered 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 stray or sheltered dogs. Among the analyzed papers 43.33% (26/60) showed five of the ten quality criteria analyzed, 16.67% (10/60) three, 15.00% (9/60) four, 10.00% (6/60) six, 6.67% (4/60) eight, only 5.00% (3/60) showed nine of the ten criteria analyzed, whereas two 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 [45.00% (27/60)] and in the last sixteen years (2003 to 2019) [81.67% (49/60)], and most of the sampled dogs were stray dogs [65.00% (39/60)]. The leptospirosis diagnostic test used more frequently was Micro Agglutination Test (MAT) [78.33% (47/60)] followed by polymerase Chain Reaction (PCR) [23.33% (14/60)], whereas the most common serovars identified were Canicola [71.43% (35/49)], Icterohaemohrragiae [65.31% (32/49)], Grippotyphosa [40.82% (20/49)] and Pomona [40.82% (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 due to the low methodologic quality of the recovered studies about leptospirosis in stray and sheltered dogs.

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

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 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 and welfare. 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 homed (Smith et al., 2019). Governments, private enterprises 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 and well-being issues.

Indeed, in animal shelters, the control of infectious diseases is a major challenge that requires a multidisciplinary approach, starting with knowledge of the epidemiologic situation of a disease and the burden it causes (Belay et al., 2017). In this context, leptospirosis, a zoonotic disease caused by *Leptospira* spp., has been an important concern, as it affects a variety of animals, including dogs and is an important zoonosis (Adler, 2015). Annually, 1.03 million people are infected and 58,900 die from leptospirosis worldwide (Costa et al., 2015), being dogs suggested as one of the main source of transmission to humans (Kurilung et al., 2019), since they can have no clinical signs of the disease despite continue shedding the bacteria in the urine (Miotto et al., 2018). The growing global number of stray and sheltered dogs makes the knowledge about the epidemiological situation of zoonotic diseases, such as leptospirosis, in these populations, crucial for the establishment of measures to mitigate the risk of infection for caretakers, future adopters and even other animals.

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

2. Material and methods

The PRISMA guidelines (Preferred Reported Items for Systematic Reviews and Meta-Analyses) were adopted in this review (Appendix S1) (http://www.prisma-statement.org/PRISMAStatement/).

2.1. Search strategy

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

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

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

Based on the guidelines for strengthening the reporting of observational studies in epidemiology (STROBE) (Vandenbroucke et al., 2007) and on representative sample 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: A - basic epidemiological requirements: 1) clearly stated objective; 2) clear definition of location where the study (town or state or country) was carried out; 3) clear definition of the period when the study was carried out; 4) clear definition of the studied population (stray or sheltered dogs); 5) clear case definition (leptospirosis-positive); B - Sampling requirements: 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 2019 (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 mean, standard deviation, median and interquartile range, when appropriated. The sampled dogs and the dogs found positives were separated by population (stray and sheltered), and a weighted average was calculated according to the sample size for all selected articles.

3. Results

3.1. Main characteristics of studies included in this systematic review

The databases search recovered 476 articles and nine were identified by active search, 404 remained after duplicates (n = 81) removal and all were published between 1973 and 2019. Title selection excluded 108 articles. From the remaining 296 articles, 144 were selected by abstract, and 152 were excluded. The full-text evaluation selected

60 cross-sectional articles for analysis of limitations and bias, whereas 84 were excluded (Fig. 1).

Geographical and temporal distribution of the articles selected by the present study are shown in the Fig. 2. The majority of the selected studies was conducted in Brazil [23.33% (14/60)], followed by Mexico [13.33% (8/60)], Malaysia [6.66% (4/60)], India [5.00% (3/60)], Italy [5.00% (3/60)] and Romania [5.00% (3/60)]. Nepal, Serbia, South Africa and Taiwan were represented each by two (3.33%) of the selected articles. The countries with only one (1.67%) study selected were Algeria, Australia, Barbados, Colombia, Egypt, Indian Ocean Island (Mayotte Island, France), Iran, Japan, Korea, Philippines, Puerto Rico, Reunion Island, Thailand, Trinidad and Tobago, Turkey, United States of America and Venezuela (Fig. 2A).

The distribution of the year when the papers were published showed that 45.00% (27/60) were published between 2013 and 2019, 36.66% (22/60) between 2003 and 2012, 10.00% (6/60) between 1973 and 1982, 8.33% (5/60) between 1993 and 2002, and none of the selected articles was published between 1983 and 1992 (Fig. 2B). The main characteristics of studies included in this systematic review are shown in Table 1.

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

The analysis of the methodological quality of the articles showed that 43.33% (26/60) exhibit five of the ten quality criteria analyzed, 16.67% (10/60) three, 15.00% (9/60) four, 10.00% (6/60) six, 6.67% (4/60) eight and only 5.00% (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 (Table 2). The final score of articles by methodological quality varied between 2 and 9, with mean and median of 5, standard deviation of 1.66 and interquartile range of 1.

All articles described the location where the study was conducted (city, town, municipality, county, state, province, or country), however six of them (10%) reported only the country. Similarly, all articles specified whether the study population. For the other criteria, 10.00% (6/60) did not inform a clear objective, only 51.67% (31/60) described the period when the research was conducted and 11.67% (7/60) did not exhibit a clear case definition (Table 2).

For the evaluation of parameters regarding the sampling adopted, only 10.00% (6/60) of articles exhibited four of the five the criteria assessed (referenced or 50% prevalence, estimated dog population, level of confidence and statistic error). Only one article (Tuemmers et al., 2013) (1.67%) used 50% prevalence, a level of confidence and a formula to estimate the sample size for determine the prevalence for an infinite population, however did not show an error value for the calculation of the sample size. Interestingly, none of the sixty selected articles specified the methodology used to randomize sample collection, neither in the eight articles that used the word “random”.

Twenty (33.33%) of the papers were published in journals without an impact factor and among all the journals that had an impact factor, the average impact factor found was 1.262, with maximum impact factor of 4.728 and minimum of 0.234.

3.3. Epidemiological situation of leptospirosis among stray and sheltered dogs

The range of the sample size of the analyzed cross-sectional studies varied from 8 to 1,615 stray or sheltered dogs (mean of 282.74, standard deviation of 364.76, median of 135 and interquartile range of 273) (Table 3). The number of *Leptospira* spp.-positive dogs per study, among the selected ones, varied from 0 to 351 (mean of 52.25, standard deviation of 70.48, median of 30 and interquartile range of 55), with a relative frequency of positives varying from 0.00% to 100.00% of the total of dogs (mean of 27.63%, standard deviation of 26.48%, median of 20% and interquartile range of 30.71%) (Table 3). In three articles (Dharanesh et al., 2009; Roach et al., 2010; Thakur, 2014), it was not possible to separate the sampled population, or the number of positive animals, or both information, considering the population of dogs of interest (stray 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 whether positive animals in Polymerase Chain Reaction (PCR) from blood were also PCR-positive from urine and MAT. The distribution of sampled and test-positive dogs according to the population (stray or sheltered) are shown in Table 3.

Among all the selected papers, the most common diagnostic test used to determine the frequency of leptospirosis in dogs was MAT [78.33% (47/60)]. The second most frequent was standard PCR [23.33% (14/60)], followed by isolation and culture [6.67% (4/60)], different types of Enzyme-Linked Immunosorbent Assays (ELISA) [6.67% (4/60)] and qPCR (quantitative PCR) [5.00% (3/60)]. Other tests (Sequencing, Pulse-Field Gel Electrophoresis, Schuffner-Mochtar's agglutination-lysis test, Urine Examination by Dark Field microscopy and Rapid Test Method) were used in 13.33% (8/60) of the studies (Fig.4 A). For the articles that performed MAT or Schuffner-Mochtar's agglutination-lysis test (precursor of MAT) and exhibited at least one positive animal, the most common serovar found was Canicola [71.43% (35/49)], followed by Icterohaemorrhagiae [65.31% (32/49)], Grippotyphosa [40.82% (20/49)], Pomona [40.82% (20/49)], Pyrogenes [28.57% (14/49)], Autumnalis [22.45% (11/49)] and others [38.78% (19/49)] (Fig.4 B). Among these studies, 100% of the tested dogs were seropositive to serovar Canicola in three studies (Cruz-Romero, 2013; Manić et al., 2014; Medina et al., 2010), whereas in one study each 100% of the tested dogs were seropositive to serovar Bataviae (Khor et al., 2016) Hardjo (Medina et al., 2010) and Icterohaemohrragiae (Medina et al., 2010) (Appendix S4).

The vaccination status against leptospirosis among the sampled dogs was informed by 26.67% (16/60) (Table 1) 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 stray or / and sheltered dogs from the other sampled animals and thereby was excluded from 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.

Analysis of risk factors related to the occurrence of leptospirosis was carried out by only 23.33% (14/60) of the selected articles (Table 1). 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 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), and dogs that lived in urban areas, sharing 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 is shown in Table 5.

4. Discussion

The comprehension of the epidemiological situation of a disease in stray and sheltered 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 stray and sheltered dogs. However, due to methodological problems found in the systematic review of the papers that addressed the subject, the real situation of this important zoonosis still remains to be determined in these animal subpopulations. Additionally, the results obtained in this systematic review 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. Nevertheless, it is important to mention that the determination of the canine 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 articles, although have performed cross-sectional studies, were focused in assessing diagnostic tests or isolate and characterizing *Leptospira* spp. strains circulating among the stray and sheltered dogs. Notwithstanding, 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 significantly represent stray or sheltered dog populations, by not following basic epidemiological criteria to perform sampling (Thrusfield, 2007; Vandenbroucke et al., 2007) (Table 2). The underrepresentation of sampling compromised the validity of the data generated (Patino & Ferreira, 2018) and prevented a meta-analysis to recalculate the prevalence of leptospirosis for these dog subpopulations. The correct method for estimating the prevalence of a disease is to conduct a representative sampling of the target population (Sedgwick, 2014), which can be performed considering the population as infinite (1), as finite (2) or performing a census (3) (Bloch & Coutinho, 2009; Thrusfield, 2007). The criteria used to evaluate the methodology of the recovered articles were those recommended for high quality cross-sectional studies (Vandenbroucke et al., 2007), allowing inferences on the produced data and epidemiological knowledge about a disease. For the studies involving stray dogs, the absence of a representative sampling may be partly justified by the difficulty to estimate this population in most locations, or it may also be due to the difficulty to find these animals that have no restrictions of movement. Nonetheless, several recovered papers also failed to describe basic aspects of scientific and epidemiological studies, beyond non-representative sampling, such as not state a clear objective, or the locations and relevant dates for the study. This low methodology quality among the selected articles probably explains the low impact factor of the journals in which these studies were published (Table 2). In fact, the exceptions to the low impact factors were observed in ten articles published in journals with impact factor greater than 2.

The majority of selected articles were published in the last sixteen years and in developing countries, such as Brazil, Mexico and Malaysia (Fig. 2), probably due to the increase of stray dogs population in these developing countries (Jackman & Rowan, 2007) and the importance that dogs have in the maintenance of leptospirosis (Macpherson et al., 2000). Brazil was the country where most of the recovered studies were conducted, presumably because of the high number of dogs (52.2 million) in the country, which is the second worldwide in number of this domestic animal (IBGE, 2013). The second country with the large number of recovered studies was Mexico, which may be associated to the great stray dog population found in the country, estimated in 16.1 million of animals (Cortez-Aguirre et al., 2018).

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

Another important information that could be extracted from the selected articles was the most common *Leptospira* serovars observed among seropositive dogs. Not surprisingly, serovar Canicola was the most frequent, probably because dogs are the reservoir of this serovar, not showing clinical signs of the disease when infected (Adler & de la Pena Moctezuma, 2010). Indeed, no signs of acute leptospirosis was reported in most of the selected papers. Subsequently, the following most common serovars were Icterohaemohrragiae, Grippotyphosa and Pomona, already described as a concern for dogs worldwide (Ellis, 2015). Moreover, the 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 it has been considered in the United States for domestic dogs since 2001 and in Europe (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 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. 4) also call for attention on the importance of these four *Leptospira* spp. 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. has a difficult 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 stray and sheltered dogs and indicate the urgent need to conduct well-designed studies in this regard to understand the epidemiological situation of the disease in these subpopulations. However, despite the low methodological quality of the recovered cross-sectional studies, the findings also showed that leptospirosis is present worldwide among stray and sheltered dogs, constituting an important threat to human and animal health.

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Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

Conflict of Interest Statement

The authors declare no conflict of interest.

Author Contributions

Conception and design: ACTRBC, CRP and EMSD. Acquisition, analysis and interpretation of data: ACTRBC and CRP. Analysis of data: RABC. Drafting the manuscript: ACTRBC and EMSD. Revising critically the manuscript: MBH, APL and EMSD. All authors reviewed and approved the final version of the manuscript.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

Adesiyun, A. A., Hull-Jackson, C., Mootoo, N., Halsall, S., Bennett, R., Clarke, N. R., Seepersadsingh, N. (2006). Seroepidemiology of canine leptospirosis in Trinidad: serovars, implications for vaccination and public health. *Journal of Veterinary Medicine. Series B, 53*(2), 91-99. doi:10.1111/j.1439-0450.2006.00922.x

Adler, B. (2015). *Leptospira and Leptospirosis* (Vol. 387): Springer-Verlag Berlin Heidelberg. doi:10.1007/978-3-662-45059-8

Adler, B., & de la Pena Moctezuma, A. (2010). Leptospira and leptospirosis. *Veterinary Microbiology, 140*(3-4), 287-296. doi:10.1016/j.vetmic.2009.03.012

Baraitareanu, S., Gurau, M. R., & Danes, D. (2014). *Survey of Leptospira spp. in captive street dogs housed in shelters*. Paper presented at the Advances in Environmental Technology and Biotechnology, Wisconsin.

Baraitareanu, S., Stanca, L. E., Gurau, M. R., & Danes, D. (2019). Research on the Leptospira infection prevalence in stray dogs in a Romanian Shelter. *Revista Romana de Medicina Veterinara, 29*(1), 37-41. Retrieved from http://agmv.ro/wp-content/uploads/2019/10/RESEARCH.pdf

Batista, C. d. S. A., Azevedo, S. S. d., Alves, C. J., Vasconcellos, S. A., Morais, Z. M. d., Clementino, I. J., Araújo Neto, J. O. d. (2004). Soroprevalência de leptospirose em cães errantes da cidade de Patos, Estado da Paraíba, Brasil. [Seroprevalence of leptospirosis in stray dogs from Patos city, state of Paraíba, Brazil]. *Brazilian Journal of Veterinary Research and Animal Science, 41*(2), 131-136. doi:10.1590/S1413-95962004000200009

Belay, E. D., Kile, J. C., Hall, A. J., Barton-Behravesh, C., Parsons, M. B., Salyer, S., & Walke, H. (2017). Zoonotic Disease Programs for Enhancing Global Health Security. *Emerging Infectious Diseases, 23*(13). doi:10.3201/eid2313.170544

Belitardo, D. R., Freitas, J. C. d., & Müller, E. E. (2000). Prevalence of leptospirosis in animals from Biologic Science Center of Londrina State University. *Semina: Ciências Agrárias (Londrina), 21*(1), 19-25.

Benacer, D., Thong, K. L., Ooi, P. T., Souris, M., Lewis, J. W., Ahmed, A. A., & Mohd Zain, S. N. (2017). Serological and molecular identification of Leptospira spp. in swine and stray dogs from Malaysia. *Tropical Biomedicine, 34*(1), 89-97.

Retrieved from https://drive.google.com/file/d/0B75lcx0mfp2OMWJWckltbTBGd3M/view

Benitez, A., Rodrigues, G. G., Gonçalves, D. D., Burke, J. C., Alves, L. A., Müller, E. E., & Freitas, J. C. d. (2010). Leptospirosis in stray dogs found in university campus: serological evaluation and urine direct exam. *Semina: Ciências Agrárias (Londrina), 31*(1), 191-196. Retrieved from http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/4907/4366

Blazius, R. D., Romao, P. R., Blazius, E. M., & Silva, O. S. d. (2005). Occurrence of Leptospira spp. soropositive stray dogs in Itapema, Santa Catarina, Brazil. *Cadernos de Saude Publica, 21*(6), 1952-1956. doi:10.1590/s0102-311x2005000600046

Bloch, K. V., & Coutinho, E. d. S. F. (2009). Fundamentos da pesquisa epidemiológica. In Medronho R. A. (Ed.), *Epidemiologia* (2nd ed., pp. 173 - 180). São Paulo: Editora Atheneu.

Bögel, K., Frucht, K., Drysdale, G., Remfry, J., World Health Organization Veterinary Public Health Unit & World Society for the Protection of Animals. (1990). Guidelines for dog population management / preparation. Initiated by K. Bögel ; editing co-ordinated by Karl Frucht, George Drysdale and Jenny Remfry. In. Geneva: World Health Organization.

Cabral, F. G. d. S., & Savalli, C. (2020). Sobre a relação humano-cão. *Psicologia USP, 31*. doi:https://doi.org/10.1590/0103-6564e190109

Chetta, M., Vicari, D., Agnello, S., Percipalle, M., Ferrantelli, V., & Vitale, M. (2014). Canine leptospirosis cases and molecular screening for Leptospira interrogans infection. *Pakistan Veterinary Journal, 34*(2), 260-262. Retrieved from http://www.pvj.com.pk/pdf-files/34_2/260-262.pdf

Chou, C., Yeh, T., Lu, Y., Shih, W., Chang, C., Chien, C., Liao, M. (2014). Prevalence of zoonotic pathogens by molecular detection in stray dogs in central Taiwan. *Thai Journal of Veterinary Medicine, 44*(3), 363-375. Retrieved from http://www.tci-thaijo.org/index.php/tjvm

Cortez-Aguirre, G. R., Jiménez-Coello, M., Gutiérrez-Blanco, E., & Ortega-Pacheco, A. (2018). Stray Dog Population in a City of Southern Mexico and Its Impact on the Contamination of Public Areas. *Veterinary Medicine International, 2018*, 1-6. doi:10.1155/2018/2381583

Costa, F., Hagan, J. E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M. S., Ko, A. I. (2015). Global Morbidity and Mortality of Leptospirosis: A Systematic Review. *PLoS Neglected Tropical Disease, 9*(9), e0003898. doi:10.1371/journal.pntd.0003898

Cruz-Romero, A., Ramero-Salas, D., Aguirre, C. A., Dominguez, M. A. & Bautista-Piña, C. (2013). Frequency of canine leptospirosis in dog shelters in Veracruz, Mexico. *African Journal of Microbiology Research*. doi:10.5897/

Desvars, A., Naze, F., Benneveau, A., Cardinale, E., & Michault, A. (2013). Endemicity of leptospirosis in domestic and wild animal species from Reunion Island (Indian Ocean). *Epidemiology and Infection, 141*(6), 1154-1165. doi:10.1017/S0950268812002075

Desvars, A., Naze, F., Vourc'h, G., Cardinale, E., Picardeau, M., Michault, A., & Bourhy, P. (2012). Similarities in Leptospira serogroup and species distribution in animals and humans in the Indian ocean island of Mayotte. *American Journal* *of Tropical Medicine and Hygiene, 87*(1), 134-140. doi:10.4269/ajtmh.2012.12-0102

Dharanesh, C. D., Suryanarayan, T., Veeregowda, B. M., Rathnamma, D., Gajendragad, M. R., Prabhudas, K., & Yathiraj, S. (2009). Seroprevalence of leptospirosis in dogs from Bangalore. *Indian Journal of Animal Sciences, 79*(2), 159-160.

Ellis, W. A. (2010). Control of canine leptospirosis in Europe: time for a change? *Veterinary Record, 167*(16), 602-605. doi:10.1136/vr.c4965

Ellis, W. A. (2015). Animal leptospirosis. In B. Adler (Ed.), *Leptospira and Leptospirosis. Current Topics Microbiology and Immunology* (1th ed., Vol. 387, pp. 99-137): Springer-Verlag Berlin Heidelberg.

FAO, Food and Agricuture Organization. (2014). *Dog population management - Report of the FAO/WSPA/IZSAM expert meeting - Banna, Italy, 14-19 March 2011.* Retrieved from Rome:

Farrington, N. P., & Sulzer, K. R. (1982). Canine leptospirosis in Puerto Rico. *International Journal of Zoonoses, 9*(1), 45-50.

Feng, T., Chou, C., Yeh, T., Su, Y., Lu, Y., Shih, W., Liao, M. (2015). Molecular prevalence of zoonotic pathogens in pet and stray dogs in southern Taiwan. *Thai Journal of Veterinary Medicine, 45*(4), 509-522. Retrieved from http://www.tci-thaijo.org/index.php/tjvm/article/view/43520/36232

Fonzar, U. J. V., & Langoni, H. (2012). Geographic analysis on the occurrence of human and canine leptospirosis in the city of Maringá, state of Paraná, Brazil. [Análise geográfica da ocorrência da leptospirose em humanos e em cães na cidade de Maringá, Paraná, Brasil]. *Revista da Sociedade Brasileira de Medicina Tropical, 45*(1), 100-105. doi:10.1590/S0037-86822012000100019

Gagnier, J., Riley, D., Altman, D., Moher, D., Sox, H., Kienle, G., & Koch, C. (2013). The CARE Guidelines: Consensus-Based Clinical Case Reporting Guideline Development. *Deutsches Ärzteblatt International, 110*, 603-608. doi:10.3238/arztebl.2013.0603

Goh, S. H., Ismail, R., Lau, S. F., Rani, P. A. M. A., Mohidin, T. B. M., Daud, F., Khor, K. H. (2019). Risk factors and prediction of leptospiral seropositivity among dogs and dog handlers in Malaysia. *International Journal of Environmental Research and Public Health, 16*(9). doi:10.3390/ijerph16091499

Gonçalez, C. C., Paes, A. C., Langoni, H., da Silva, R. C., Greca, H., Camossi, L. G., Guimarães, F. F., Ullmann, L. S. (2010). Antibodies to Leptospira spp., Toxoplasma gondii, and Neospora caninum in mongrel dogs kept in private kennel. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62(4),*1011-1014. doi: 10.1590/S0102-09352010000400037

Hafemann, D. C. M., Merlini, L. S., Gonçalves, D. D., Fortes, M. S., Navarro, I. T., Chiderolli, R. T., Freitas, J. C., Gonçalves, A. P. P., Rosa, G., Sposito, P. H. (2018). Detection of anti-Leptospira spp., anti-Brucella spp., and anti-Toxoplasma gondii antibodies in stray dogs. *Semina: Ciências Agrárias(Londrina)*, *39(1),*167-173. Retrived from: http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/27141/23051

Hart, L. A., & Yamamoto, M. (2016). Dogs as helping partners and companions for humans. 247-270. doi:10.1017/9781139161800.013

Hughes, J., & Macdonald, D. W. (2013). A review of the interactions between free-roaming domestic dogs and wildlife. *Biological Conservation, 157*, 341-351. doi:10.1016/j.biocon.2012.07.005

IBGE, Instituto Brasileiro de Geografia e Estatistica. (2013). População de animais de estimação no Brasil [Pet population in Brazil] Retrieved from https://www.gov.br/agricultura/pt-br/assuntos/camaras-setoriais-tematicas/documentos/camaras-tematicas/insumos-agropecuarios/anos-anteriores/ibge-populacao-de-animais-de-estimacao-no-brasil-2013-abinpet-79.pdf/view

Ivana, S., Bogdan, A. T., Ipate, I., Andreescu, N., Chiurciu, C., Chiurciu, V., Caplan, D.M., Bărăităreanu, S., Ţogoe, I., Popescu, A. N. (2010). Biodiversity of the germs involved in the human and animal leptospirosis in stray dog of Bucharest temporally adopted by the community. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine,67(1),*82-86. Retrieved from: http://journals.usamvcj.ro/veterinary

Jackman, J., & Rowan, A. (2007). Free-roaming dogs in developing countries: The benefits of capture, neuter, and return programs. *The State of the Animals IV: 2007*, 55-78.

Jimenez-Coello, M., Ortega-Pacheco, A., Guzman-Marin, E., Guiris-Andrade, D. M., Martinez-Figueroa, L., Acosta-Viana, K. Y. (2010). Stray dogs as reservoirs of the zoonotic agents Leptospira interrogans, Trypanosoma cruzi, and Aspergillus spp. in an urban area of Chiapas in southern Mexico. *Vector-Borne and Zoonotic Disease,10(2),*135-141. doi: 10.1089/vbz.2008.0170

Jimenez-Coello, M., Vado-Solis, I., Cárdenas-Marrufo, M. F., Rodríguez-Buenfil, J. C., Ortega-Pacheco, A. (2008). Serological survey of canine leptospirosis in the tropics of Yucatan Mexico using two different tests. *Acta Tropica, 106(1),*22-26. doi: 10.1016/j.actatropica.2007.12.011

Jittapalapong, S., Sittisan, P., Sakpuaram, T., Kabeya, H., Maruyama, S., Inpankaew, T. (2009). Coinfection of Leptospira spp. and Toxoplasma gondii among stray dogs in Bangkok, Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health, 40(2)*, 247-252. Retrieved from: http://www.tm.mahidol.ac.th/en/seameo/publication.htm

Jung, B. Y., Song, Y. K., Choi, J. S., Lee, K. W., Ha, T. Y., Yoon, S. S., Kweon, C. H., Park, C. K. (2008). Seroprevalence of leptospirosis in animals in Korea. *Veterinary Record, 163(1),* 28-19. Retrieved from: http://veterinaryrecord.bvapublications.com/archive/

Khamesipour, F., Doosti, A., Emadi, M. F., & Awosile, B. (2014). Detection of Brucella sp and Leptospira sp in dogs using conventional polymerase chain reaction. *Bulletin of the Veterinary Institute in Pulawy, 58*(4), 527-531. doi:10.2478/bvip-2014-0081

Khor, K. H., Tan, W. X., Lau, S. F., Mohd, A. R., Rozanaliza, R., Siti, K. B., & Abdul, R. B. (2016). Seroprevalence and molecular detection of leptospirosis from a dog shelter. *Tropical Biomedicine, 33*(2), 276-284. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-84978808763&partnerID=40&md5=8b549de76fb31e54c4a5e49de0085375

Kumar, A., Sinha, D. K., Chaudhury, P., Shankar, H., & Srivastava, S. K. (2009). Comparative studies on seroepidemiology of canine leptospirosis by micro agglutination test (MAT) and recombinant Lip L32 ELISA. *Indian Journal of Animal Sciences, 79*(11), 1089-1094. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-74549114848&partnerID=40&md5=34c120bb37d9daa133c21bcada3faa9c

Kurilung, A., Keeratipusana, C., Suriyaphol, P., Hampson, D. J., & Prapasarakul, N. (2019). Genomic analysis of Leptospira interrogans serovar Paidjan and Dadas isolates from carrier dogs and comparative genomic analysis to detect genes under positive selection. *BMC Genomics, 20*(1), 168. doi:10.1186/s12864-019-5562-z

Lau, S. F., Wong, J. Y., Khor, K. H., Roslan, M. A., Abdul Rahman, M. S., Bejo, S. K., Bahaman, A. R. (2017). Seroprevalence of Leptospirosis in Working Dogs. *Topics in Companion Animal Medicine, 32*(4), 121-125. doi:10.1053/j.tcam.2017.12.001

Macpherson, C. N. L., Meslin, F. X., & Wandeler, A. I. (2000). *Dogs, zoonoses and Public Health*. Wallingford, Oxon, UK.: CABI Publishing. doi:10.1079/9780851994369.0000

Mamak, N., Ozkanlar, Y., Kazim-Borku, M., & Yilmaz-Imren, H. (2014). Detection of antibodies against Leptospira spp. and Listeria monocytogenes, and intestinal parasite eggs in Kangal dogs. *Revista Cientifica de la Facultad de Ciencias Veterinarias de la Universidad del Zulia, 24*(1), 18-23. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-84902002952&partnerID=40&md5=d2b3d50bffd72503fe59eee11f28ad95

Manić, M., Prokić, N., Gojković, K., Đorić, G., Vasić, A., Marić, J., Đuričić, B. (2014). Seroprevalence of some infectious diseases in stray dogs in the wider territory of Leskovac city. *Arhiv Veterinarski Medicine, 7*(1), 19-27. Retrieved from http://niv.ns.ac.rs/?page_id=557

Mbilo, C., Coetzer, A., Bonfoh, B., Angot, A., Bebay, C., Cassamá, B., Zinsstag, J. (2020). Dog rabies control in West and Central Africa: A review. *Acta tropica*, 105459. doi:10.1016/j.actatropica.2020.105459

Medina, A. Z., Guerra, B. M., & Veliz, N. (2010). Serological study of leptospirosis in canines from a shelter in the state of Aragua. *Revista de la Facultad de Ciencias Veterinarias, Universidad Central de Venezuela, 51*(2), 93-97. Retrieved from http://www.scielo.org.ve/scielo.php?script=sci_arttext&pid=S0258-65762010000200004&lng=en&nrm=iso&tlng=es

Meira, C. D., Wenceslau, A. A., Carvalho, F. S., Albuquerque, G. R., & Dias, R. C. (2011). Molecular diagnosis of leptospirosis in blood of dogs naturally infected. *Revista Brasileira de Medicina Veterinaria, 33*(1), 7-11. Retrieved from http://www.somverj.org.br

Merien, F., Amouriaux, P., Perolat, P., Baranton, G., & Saint Girons, I. (1992). Polymerase Chain Reaction for Detection of Leptospira spp. in Clinical Samples. Journal of Clinical Microbiology*, 30*(9), 2219-2224.

Miotto, B. A., Guilloux, A. G. A., Tozzi, B. F., Moreno, L. Z., Da Hora, A. S., Dias, R. A., Hagiwara, M. K. (2018). Prospective study of canine leptospirosis in shelter and stray dog populations: Identification of chronic carriers and different Leptospira species infecting dogs. *PLoS ONE, 13*(7). doi:10.1371/journal.pone.0200384

Mohammed, H., Nozha, C., Hakim, K., & Abdelaziz, F. (2011). Leptospira: Morphology, Classification and Pathogenesis. *Journal of Bacteriology & Parasitology, 02*(06). doi:10.4172/2155-9597.1000120

Myburgh, J. G., Posnett, S. J., & Lawrence, J. V. (1993). Serological survey for canine leptospirosis in the Pretoria area. *Journal of the South African Veterinary Association, 64*(1), 37-38.

OIE, World Organization for Animal Health. (2012). Manual of Diagnostic Tests and Vaccines for terrestrial animals (mammals, birds and bees). *Manual of Standards for Diagnostic Tests and Vaccines, III*.

Ojha, K. C., Singh, D. K., Kaphle, K., Shah, Y., & Pant, D. K. (2018). Sero-prevalence of leptospirosis and differentiation in blood parameters between positive and negative cases in dogs of Kathmandu Valley. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 112*(8), 378-382. doi:10.1093/trstmh/try065

Oliveira, S. T., Messick, J. B., Biondo, A. W., dos Santos, A. P., Stedile, R., Dalmolin, M. L., Gonzalez, F. H. D. (2012). Exposure to Leptospira spp. in Sick Dogs, Shelter Dogs and Dogs from an Endemic Area: Points to Consider. *Acta Scientiae Veterinariae, 40*(3). Retrieved from <Go to ISI>://WOS:000307755500011

Ortega-Pacheco, A., Colin-Flores, R. F., Gutierrez-Blanco, E., & Jimenez-Coello, M. (2008). Frequency and type of renal lesions in dogs naturally infected with leptospira species. *Annals The New York Academy of Sciences, 1149*, 270-274. doi:10.1196/annals.1428.088

Patino, C. M., & Ferreira, J. C. (2018). Internal and external validity: can you apply research study results to your patients? *Jornal Brasileiro Pneumologia, 44*(3), 183. doi:10.1590/s1806-37562018000000164

Paz, G. S. d., Rocha, K. S., Lima, M. S., Jorge, E. M., Pantoja, J. C. F., de Moraes, C. C. G., & Langoni, H. (2015). Seroprevalence for brucellosis and leptospirosis in dogs from Belém and Castanhal, State of Pará, Brazil. *Acta Amazonica, 45*(3), 265-270. doi:10.1590/1809-4392201403486

Rivera Flores, A., Roa Riol, M. d. l. Á., Ordóñez Badillo, M. L., & Peña Moctezuma, A. d. l. (1999). Seroprevalence of leptospirosis in stray dogs in northern Mexico city. *Veterinaria México, 30*(1), 105-107.

Roach, J. M., van Vuuren, M., & Picard, J. A. (2010). A serological survey of antibodies to Leptospira species in dogs in South Africa. *Journal of South African Veterinary Association, 81*(3), 156-159. doi:10.4102/jsava.v81i3.139

Rodríguez, A. L., Ferro, B. E., Varona, M. X., & Santafé, M. (2004). Exposure to Leptospira in stray dogs in the city of Cali. *Biomédica : revista del Instituto Nacional de Salud, 24*(3), 291-295. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-16544390971&partnerID=40&md5=212aaeb1ca8f1afd28a7667b4e34e683

Ryu, E. (1975). An investigation of canine antileptospiral antibodies in Japan. *The International Journal of Zoonoses, 2*(1), 16-34.

Scanziani, E., Origgi, F., Giusti, A. M., Iacchia, G., Vasino, A., Pirovano, G., Tagliabue, S. (2002). Serological survey of leptospiral infection in kennelled dogs in Italy. *Journal of Small Animal Practice, 43*(4), 154-157. doi:10.1111/j.1748-5827.2002.tb00048.x

Schuller, S., Francey, T., Hartmann, K., Hugonnard, M., Kohn, B., Nally, J. E., & Sykes, J. (2015). European consensus statement on leptospirosis in dogs and cats. *Journal of Small Animal Practice, 56*(3), 159-179. doi:10.1111/jsap.12328

Sedgwick, P. (2014). Cross sectional studies: advantages and disadvantages. *BMJ, 348*(mar26 2), g2276-g2276. doi:10.1136/bmj.g2276

Segovia, P. T., Blum Domínguez, S. D. C., Chi Dzib, M. Y., Velázquez, M. G. M., Nuñez Oreza, L. A., Gómez Solano, M. I., & Caballero Poot, R. I. (2013). Detection of reactive canines to leptospira in campeche city, Mexico. *Revista Argentina de Microbiologia, 45*(1), 34-38. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-84876053570&partnerID=40&md5=e67ab4a23e91cc8c6c321aa7b3344a8f

Senthil, N. R., Palanivel, K. M., & Rishikesavan, R. (2013). Seroprevalence of Leptospiral Antibodies in Canine Population in and around Namakkal. *Journal Veteterinary Medicine, 2013*, 971810. doi:10.1155/2013/971810

Siam, M. A., Karim, A. M. A., Hamed, O. M., & Zakaria, A. (1973). The Possible Role of Stray Dogs and Cats in the Epidemiology of some Bacterial Human Pathogens in Egypt. *Zentralblatt für Veterinärmedizin Reihe B, 20*(6), 409-414. doi:10.1111/j.1439-0450.1973.tb01144.x

Silva, E. R. D. F. S., Castro, V., Prianti, M. G., Gonçalves, L. M. F., Sobrinho Júnior, E. P. C., Drumond, K. O., & Mineiro, A. L. B. B. (2017). Occurrence of antibodies against Leptospira spp in dogs from Teresina, Piauí, Brazil. *Brazilian Journal of Veterinary Research and Animal Science, 54*(1), 88-91. doi:10.11606/issn.1678-4456.bjvras.2017.110588

Smith, L. M., Hartmann, S., Munteanu, A. M., Dalla Villa, P., Quinnell, R. J., & Collins, L. M. (2019). The Effectiveness of Dog Population Management: A Systematic Review. *Animals (Basel), 9*(12). doi:10.3390/ani9121020

Thakur, M. (2014). Sero-prevalence of leptospiral infection in canine population of Kathmandu valley. *Nepalese Journal of Agricultural Sciences, 12*, 67-73.

Retrieved from http://hicast.edu.np/file/file_down/pdf/pdf11/hx1JAeNJAS_2014_12.pdf

Thiermann, A. B. (1980). Canine leptospirosis in Detroit. *American Journal of Veterinary Research, 41*(10), 1659-1661.

Thrusfield, M. (2007). *Veterinary Epidemiology* (3 th ed.). Oxford. UK: Blackwell Science Ltd. Retrieved from https://books.google.com.br/books?id=LZfevagYF4YC

Tuemmers, C., Lüders, C., Rojas, C., Serr¡, M., Espinoza, R., & Castillo, C. (2013). Prevalencia de leptospirosis en perros vagos capturados en la ciudad de Temuco, 2011. [Prevalence of leptospirosis in vague dogs captured in Temuco city, 2011]. *Revista Chilena de Infectología, 30*(3), 252-257. doi:10.4067/S0716-10182013000300003

Vandenbroucke, J. P., von Elm, E., Altman, D. G., Gøtzsche, P. C., Mulrow, C. D., Pocock, S. J., Initiative, S. (2007). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Medicine, 4*(10), e297-e297. doi:10.1371/journal.pmed.0040297

Vicari, D., Percipalle, M., Concetta, L. M., Li Vecchi, L., Curro, V., Vitale, M., & Vicenzo, F. (2007). Evidencia de leptospirosis canina en las perreras de Sicilia según el método de RCP. [Evidence of canine leptospirosis in Kennels in Sicily, by PCR method]. *Revista Cubana de Medicina Tropical, 59*(1). Retrieved from http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0375-07602007000100012&lang=pt

Villanueva, S. Y. A. M., Baterna, R. A., Cavinta, L. L., Yanagihara, Y., Gloriani, N. G., & Yoshida, S. i. (2018). Seroprevalence of leptospirosis among water buffaloes, pigs, and dogs in selected areas in the Philippines, 2007 to 2008. *Acta Medica Philippina, 52*(1), 109-117. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-85047389833&partnerID=40&md5=12c2dc6125b81bd531eb4094acf587a1

Vojinović, D., Bogićević, N., Vasić, A., Manić, M., Radovanović, M. E., Rogožarski, D., Valčić, M. (2015). Seroepidemiological survey of leptospiral infection in stray dogs in Serbia. *Turkish Journal of Veterinary and Animal Sciences, 39*(6), 719-723. Retrieved from http://journals.tubitak.gov.tr/veterinary/

Weekes, C. C., Everard, C. O. R., & Levett, P. N. (1997). Seroepidemiology of canine leptospirosis on the island of Barbados. *Veterinary Microbiology, 57*(2/3), 215-222. doi:10.1016/S0378-1135(97)00127-2

WSPA, World Sociaty for the Protection of Animals. (2011). Working with strays: a humane alternative. [Website] Retrieved from http://www.wspa-usa.org/wspaswork/dogs/companionanimals/ahumanealternative.html

Yasuda, P. H., Santa Rosa, C. A., Myers, D. M., & Yanaguita, R. M. (1980a). The isolation of leptospires from stray dogs in the city of Sao Paulo, Brazil. *International Journal of Zoonoses, 7*(2), 131-134.

Yasuda, P. H., Santa Rosa, C. A., & Yanaguita, R. M. (1980b). Variação sazonal na prevalência de leptospirose em cães de rua da cidade de São Paulo, Brasil. [Seasonal variation in the prevalence of leptospirosis in stray dogs in the citv of S. Paulo (Brazil)]. *Revista de Saúde Pública, 14*(4), 589-596. doi:10.1590/S0034-89101980000400014

Zaidi, S., Bouam, A., Bessas, A., Hezil, D., Ghaoui, H., Ait-Oudhia, K., Bitam, I. (2018). Urinary shedding of pathogenic Leptospira in stray dogs and cats, Algiers: a prospective study. *PLoS ONE, 13*(5), e0197068. doi:10.1371/journal.pone.0197068

Ziehl-Quirós, E. C., García-Aguilar, M. C., & Mellink, E. (2017). Colony-level assessment of Brucella and Leptospira in the Guadalupe fur seal, Isla Guadalupe, Mexico. *Diseases of Aquatic Organisms, 122*(3), 185-193. doi:10.3354/dao03073

Zwijnenberg, R. J. G., Smythe, L. D., Symonds, M. L., Dohnt, M. F., & Toribio, J. A. L. M. L. (2008). Cross-sectional study of canine leptospirosis in animal shelter populations in mainland Australia. *Australian Veterinary Journal, 86*(8), 317-323. doi:10.1111/j.1751-0813.2008.00324.x

Tables

Table 1: Detailed information of the 60 cross-sectional studies selected by this systematic review on canine leptospirosis in stray or sheltered dogs, published between 1973 and 2019.

First author, Year	Town / city	State / Province	Country	Period	Year	Population	Nº sampled dogs	*Leptopira* positive dogs (%)	Diagnostic method	Diagnostic method Cut-off	Information on leptospirosis vaccination	Risk factor analysis
Adesiyun, 2006	NI	NI	Trinidad and Tobago	February to July	2005	Sheltered	113	5 (4.42)	MAT	1:100 dilution or greater was considered seropositive, 1:800 considered acute infection	No	No
Baraitareanu, 2014	Galati County, Braila County and Arges County	South-Eastern Region	Romenia	NI	NI	Sheltered	77	31 (40.26)	PCR and MAT	1:100 dilution with 50% agglutination	No	No
Baraitareanu, 2019	NI	NI	Romenia	NI	NI	Sheltered	19	18 (94.74)	PCR and MAT	NI	No	No
Batista, 2004	Patos	Paraíba	Brazil	February to April	2003	Stray	130	26 (20.00)	MAT	1:100 dilution with 50% agglutination	No	Yes
Belitardo, 2000	UEL/Londrina	Paraná	Brazil	March to September	1998 to 1999	Stray	289	110 (38.06)	MAT/DEU/Isolation	1:100 dilution with 50% agglutination	No	No
Benacer, 2017	Klang Valley in Kuala Lumpur	Selangor	Malaysia	NI	2012 to 2013	Stray	150	11 (7.33)	Isolation/PCR/MAT/ Sequencing/PFGE	NI for MAT	No	No
Benitez, 2010	UEL/Londrina	Paraná	Brazil	July to September	2007	Stray	33	7 (21.21)	MAT	1:100 dilution with 50% agglutination	Yes	No
Blazius, 2005	Itapema	Santa Catarina	Brazil	August to May	2000 to 2005	Stray	590	62 (10.51)	MAT	1:100 dilution with 50% agglutination	No	No
Chetta, 2014	Sicily	Sicily	Italy	April to March	2009 to 2010	Sheltered	183	26 (14.21)	PCR	NA	No	No
Chou, 2014	Taichung, Changhua and Yunlin County	Central region	Taiwan	August to July	2009 to 2011	Sheltered	720	52 (7.22)	PCR	NA	No	Yes
Cruz-Ramero, 2013	Veracruz	Veracruz	Mexico	NI	NI	Sheltered	92	8 (8.70)	MAT	Equal to or greater than 1:100 dilution	Yes	Yes
Desvars, 2013	NI	NI	Reunion Island	February and August	2009	Stray	50	23 (46.00)	MAT/PCR/qPCR	1:100 cut off	No	No
Desvars, 2012	Mayotte Island	Comoros Islands	Indian Ocean Island (France)	March and May	2007	Stray	8	7 (87.50)	MAT/qPCR/ Sequencing	1:100 cut off	Yes	No

Dharanesh, 2009 †	Bangalore	Karnataka	India	NI	NI	Sheltered	-	79 (-)	MAT	1:100 dilution with 50% agglutination	No	No
Farrington, 1982	Guaynabo, San Juan and Mayaguez	Metropolitan Region of San Juan-Caguas-Guaynabo, Metropolitan Region of Mayaguez	Puerto Rico	June to August	1980	Sheltered	116	73 (62.93)	MAT	1:100 dilution	No	No
Feng, 2015	Kaohsiung City and Pingtung County	Southeast region	Taiwan	August to July	2009 to 2011	Sheltered	720	0 (0.00)	PCR and Sequencing	NA	No	No
Fonzar, 2012	Maringá	Paraná	Brazil	NI	2006 to 2008	Stray	355	41 (11.55)	MAT	NI	No	No
Goh, 2019	NI	Johore and Selangor	Malaysia	5 Months	NI	Sheltered	193	42 (21.76)	MAT	1:100 dilution with 50% agglutination	Yes	Yes
Gonçalez, 2010	Avaré	São Paulo	Brazil	NI	NI	Sheltered	300	28 (9.33)	MAT	NI	No	No
Hafemann, 2018	Assis Chateaubriand, São Jorge do Patrocínio, Pérola, Umuarama, Marechal Cândido Rondon, Moreira Sales, and Paranavaí.	Paraná	Brazil	March and October	2015	Sheltered	181	30 (16.57)	MAT	1:100 dilution with 50% agglutination	No	No
Ivana, 2010	Bucharest	Muntenia region	Romania	NI	NI	Sheltered	103	38 (36.89)	MAT	1:400 dilution and 50% microscopic field were agglutinated	Yes	No
Jimenez-Coello, 2010	Tuxtla Gutierrez	Chiapas	Mexico	January to July	NI	Sheltered	224	11 (4.91)	MAT	NI	No	Yes
Jimenez-Coello, 2008	Merida	Yucatan	Mexico	NI	NI	Sheltered	400	140 (35.00)	MAT and ELISA	1:100 dilution with 50% agglutination	No	No
Jittapalapong, 2009	Bangkok	Central Region	Thailand	NI	NI	Stray	230	205 (89.13)	MAT	1:100 dilution with 50% agglutination	No	Yes
Jung, 2008	Seoul	Northwest region	Korea	October and December	2005 and 2006	Sheltered	80	6 (7.50)	MAT	1:100 dilution	No	No
Khamesipour, 2014	Isfahan and Shahrekord	Ispaão Province and Chaharmahal and Bakhtiari Province	Iran	May and December	2013	Stray	30	12 (40.00)	PCR	NA	No	Yes
Khor, 2016.	NI	Selangor	Malaysia	December	2014	Sheltered	80	3 (3.75)	MAT and PCR	1:80 dilution	Yes	No
Kumar, 2009	Delhi	National Capital Region	India	NI	NI	Stray	42	4 (9.52)	MAT and Lipl32 ELISA	1:100 dilution with 50% agglutination	Yes	No
Lau, 2017	NI	NI	Malaysia	NI	NI	Sheltered	96	3 (3.13)	MAT	1:80 dilution with >50%	Yes	No

										agglutination		
Mamak, 2014.	Kangal	Sivas	Turkey	NI	NI	Stray and Sheltered	29	2 (6.90)	MAT	NI	No	No
Manić, 2014	Leskovac	Jablanica District	Serbia	NI	NI	Stray	50	4 (8.00)	MAT	1:100 dilution	No	No
Medina 2010	Maracay	Aragua	Venezuela	NI	NI	Sheltered	30	30 (100.00)	MAT	Major or equal to 1:100 title	No	No
Meira, 2011	Ilheus	Bahia	Brazil	NI	NI	Stray	100	4 (4.00)	PCR	NA	No	Yes
Miotto, 2018	São Paulo, Mogi das Cruzes and USP/São Paulo	São Paulo	Brazil	NI	NI	Stray and Sheltered	123	54 (43.90)	PCR and MAT	1:100 dilution with 50% agglutination	Yes	No
Myburgh, 1993	Pretoria	Tshwane district	South Africa	NI	1989 to 1990	Stray	400	7 (1.75)	MAT	1:160 dilution or more	No	No
Ojha, 2018	Kathmandu, Bhaktapur and Lalitpur	Kathmandu Valley	Nepal	August to January	2016 to 2017	Stray	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.	Yes	Yes
Oliveira, 2012‡	Porto Alegre	Rio Grande do Sul	Brazil	May and February	2007 and 2009	Sheltered	65	35 (53.85)	MAT and PCR	1:100 dilution or more	No	No
Ortega-Pacheco, 2008	Merida	Yakatan	Mexico	NI	NI	Stray	350	122 (34.86)	MAT	NI	No	No
Paz, 2015	Belém and Castanhal	Pará	Brazil	NI	2009 to 2010	Sheltered	143	22 (15.38)	MAT	1:100 dilution with 50% agglutination	No	Yes
Rivera Flores, 1999	Mexico City	Federal District	Mexico	NI	NI	Sheltered	135	52 (38.52)	MAT	1:100 dilution	No	No
Roach, 2010†	NI	Provinces of Kwazulu-Natal, Eastern Cape, Western Cape and Gauteng	South Africa	NI	NI	Sheltered	-	-	MAT	1:100 dilution with 50% agglutination	No	Yes
Rodríguez, 2004	Cali	Cauca Valley	Colombia	NI	2001 to 2003	Stray	197	81 (41.12)	MAT	1:100 dilution with 50% agglutination	No	No
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	Stray	1,615	351 (21.73)	Schuffner-Mochtar's Agglutination-Lysis Test	NI	No	No
Scanziani, 2002	Milan	Lombardia region	Italy	NI	NI	Sheltered	211	71 (33.65)	MAT	1:100 dilution to all serovars and 1:800 for Canicola and Icterohaemohrragiae	Yes	No
Segovia, 2013	Campeche	Yucatan Peninsula	Mexico	NI	NI	Stray	142	38 (26.76)	MAT	1:100 dilution and presented	Yes	No

										50% agglutination or more was title with two reason dilution		
Senthil, 2013	Namakkal	Tamilnadu	India	NI	NI	Stray	176	143 (81.25)	MAT	1:40 dilution with 50% agglutination	Yes	No
Siam, 1973	Cairo and Giza	Cairo	Egypt	NI	NI	Stray	50	6 (12.00)	Schuffner-Mochtar's agglutination-lysis test	NI	No	No
Silva, 2017	Terezina	Piaui	Brazil	July to January	2010 to 2012	Sheltered	425	74 (17.41)	MAT	1:100 dilution with 50% agglutination	No	No
Thakur, 2014†	NI	Kathmandu	Nepal	April to August	NI	Stray	31	-	Ig by Rapid Test Kit Method (SD Bioline).	NA	Yes	No
Thiermann, 1980	Detroit	Michigan	United States of America	NI	NI	Stray	556	187 (33.63)	MAT	NI	No	No
Tuemmers, 2013	Temuco	Cautín province	Mexico	18 Months	2011	Stray	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.	No	Yes
Vicari, 2007.	Palermo and Agrigento	Sicily	Italy	NI	NI	Sheltered	64	5 (7.81)	PCR	NA	No	No
Villanueva, 2018	Manila, Quezon City and Makati City	Manila and Lone de Taguig City-Pateros district	Philippines	January to August	2007 to 2008	Sheltered	109	86 (78.90)	MAT and Isolation	1:80 dilution with 50% agglutination	No	No
Vojinović, 2015	NI	NI	Serbia	April to June	2010 to 2013	Sheltered	1,045	57 (5.45)	MAT	1:100 dilution	Yes	No
Weekes, 1997	NI	NI	Barbados	NI	NI	Sheltered	78	48 (61.54)	MAT	1:100 dilution	No	No
Yasuda, 1980a	São Paulo	São Paulo	Brazil	October to September	1976 to 1977	Stray	1,415	35 (2.47)	Isolation	NA	No	No
Yasuda, 1980b	São Paulo	São Paulo	Brazil	October to September.	1976 to 1977	Stray	1,428	308 (21.57)	MAT	1:100 dilution	No	No
Zaidi, 2018	Algiers	Argel province	Algeria	April to November	2017	Stray	104	5 (4.81)	qPCR/ PCR/ Sequencing	NA	No	Yes
Ziehl-Quirós, 2017	Guadalupe Fur Seal	Isla Guadalupe	Mexico	August	2014	Stray	46	12 (26.09)	MAT	1:50 dilution with 50% agglutination	No	No
Zwijnenberg, 2008	NI	Queensland, New South Weles, Western Australia and Northern Territory	Australia	NI	2004	Sheltered	956	18 (1.88)	MAT	≥1:50 or 1:100 to serovars L. interrogans sv. Copenhageni and sv. Australis	Yes	Yes

UEL: Universidade Estadual de Londrina (State University of Londrina). **USP:** Universidade de São Paulo (University of São Paulo). **MAT:** Microscopic Agglutination Test. **PCR:** Polymerase Chain Reaction. **PFGE:** Pulse Field Gel Electrophoresis. **DUE:** Direct Urine Examination in dark field. **ELISA:** Enzyme Linked Immunosorbent Assay. **Lip32 ELISA:** Enzyme Linked Immunosorbent Assay using the Lip32 protein as antigen. **qPCR:** quantitative Polymerase Chain Reaction. **Sv:** serovar. **NI:** Not Informed. **NA:** Not applicable. †: Does not separate sampled or positive results for stray or sheltered dogs from other dogs sampled. ‡: Considered only positives in MAT.

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

First author, year	Objective	Local	Period	Population	Case definition	Referenced or 50% Prevalence	Level of confidence	Estimation of dog population	Statistical error	Randomized sample	Score	Impact Factor
Adesiyun, 2006	1	1	1	1	1	0	0	0	0	0	5	NA
Baraitareanu, 2014	1	1	0	1	1	0	0	1	0	0	5	NA
Baraitareanu, 2019	1	1	0	1	1	0	0	1	0	0	5	NA
Batista, 2004	1	1	1	1	1	1	1	1	1	0	9	NA
Belitardo, 2000	1	1	1	1	1	0	0	0	0	0	5	0.412
Benacer, 2017	1	1	1	1	1	0	0	0	0	0	5	0.509
Benitez, 2010	1	1	1	1	1	0	0	0	0	0	5	0.412
Blazius, 2005	1	1	1	1	1	0	0	0	0	0	5	1.408
Chetta, 2014	1	1	1	1	1	0	0	0	0	0	5	1.175
Chou, 2014	1	1	1	1	1	0	0	1	0	0	6	0.234
Cruz-Ramero, 2013	1	1	0	1	1	0	0	0	0	0	4	NA
Desvars, 2012	1	1	1	1	1	0	0	0	0	0	5	2.152
Desvars, 2013	1	1	1	1	1	0	0	0	0	0	5	2.126
Dharanesh, 2009	0	1	0	1	1	0	0	0	0	0	3	0.278
Farrington, 1982	1	1	1	1	1	0	0	0	0	0	5	NA
Feng, 2015	1	1	1	1	1	0	0	1	0	0	6	0.234
Fonzar, 2012	1	1	1	1	1	0	0	1	0	0	6	1.339
Goh, 2019	1	1	0	1	1	0	0	0	0	0	4	2.849
Gonçalez, 2010	1	1	0	1	0	0	0	0	0	0	3	0.279
Hafemann, 2018	1	1	1	1	1	0	0	0	0	0	5	0.412
Ivana, 2010	1	1	0	1	1	0	0	0	0	0	4	NA
Jimenez-Coello, 2008	1	1	0	1	1	1	1	1	1	0	8	2.249
Jimenez-Coello, 2010	1	1	0	1	0	0	0	0	0	0	3	2.555
Jittapalapong, 2009	1	1	0	1	1	0	0	0	0	0	4	0.245
Jung, 2008	1	1	1	1	1	0	0	0	0	0	5	2.442
Khamesipour, 2014	1	1	1	1	1	0	0	0	0	0	5	NA
Khor, 2016	1	1	1	1	1	0	0	1	0	0	6	0.509
Kumar, 2009	1	1	0	1	1	0	0	0	0	0	4	0.278
Lau, 2017	1	1	0	1	1	0	0	1	0	0	5	0.983
Mamak, 2014	1	1	0	1	0	0	0	0	0	0	3	NA
Manić, 2014	0	1	0	1	1	0	0	0	0	0	3	NA
Medina 2010	1	1	0	1	1	0	0	1	0	0	5	NA
Meira, 2011	1	1	0	1	1	1	1	1	1	0	8	NA
Miotto, 2018	1	1	0	1	1	0	0	1	0	0	5	2.740
Myburgh, 1993	0	1	1	1	1	0	0	0	0	0	4	1.160
Ojha, 2018	1	1	1	1	1	0	1	1	1	0	8	1.868
Oliveira, 2012	1	1	1	1	1	0	0	0	0	0	5	0.321
Ortega-Pacheco, 2008	1	1	0	1	1	0	0	0	0	0	4	4.728
Paz, 2015	1	1	1	1	1	0	0	1	0	0	6	0.768
Rivera Flores, 1999	0	1	0	1	1	0	0	0	0	0	3	0.192

Roach, 2010	1	1	0	1	1	0	1	0	1	0	6	1.160
Rodríguez, 2004	1	1	1	1	1	0	0	0	0	0	5	NA
Ryu, 1975	0	1	0	1	0	0	0	0	0	0	2	NA
Scanziani, 2002	1	1	0	1	1	0	0	1	0	0	5	1.103
Segovia, 2013	1	1	0	1	1	1	1	1	1	0	8	1.221
Senthil, 2013	1	1	0	1	1	0	0	0	0	0	4	0.234
Siam, 1973	1	1	0	1	0	0	0	0	0	0	3	NA
Silva, 2017	1	1	1	1	1	0	0	0	0	0	5	NA
Thakur, 2014	1	1	0	1	0	0	0	0	0	;0	3	NA
Thiermann, 1980	1	1	0	1	0	0	0	0	0	0	3	0.811
Tuemmers, 2013	1	1	1	1	1	1	1	0	0	0	7	0.520
Vicari, 2007	0	1	0	1	1	0	0	0	0	0	3	NA
Villanueva, 2018	1	1	1	1	1	0	0	0	0	0	5	NA
Vojinović, 2015	1	1	1	1	1	0	0	0	0	0	5	0.552
Weekes, 1997	1	1	0	1	1	0	0	0	0	0	4	3.030
Yasuda, 1980a	1	1	1	1	1	0	0	0	0	0	5	NA
Yasuda, 1980b	1	1	1	1	1	0	0	0	0	0	5	1.748
Zaidi, 2018	1	1	1	1	1	0	0	0	0	0	5	2.740
Ziehl-Quirós, 2017	1	1	1	1	1	1	1	1	1	0	9	1.368
Zwijnenberg, 2008	1	1	1	1	1	1	1	1	1	0	9	1.145

Objective: clearly stated objective. **Local**: clear definition of location where the study (city or state or country) was carried out. **Period**: clear definition of period (year) when the study was carried out. **Population**: clear definition of the studied population (stray or sheltered dogs). **Case Definition**: clear case definition (leptospirosis-positive). **Referenced or 50% Prevalence**: a referenced or 50% prevalence was used. **Level of Confidence**: a level of confidence was adopted. **Estimation of Dog Population**: the size of sampled dog population was estimated or an infinite population was considered. **Statistical Error**: a statistic error was adopted. **Randomized Sample**: the sampling performed was randomized or all animas in the population were sampled (census). **Score**: It is the sum of all information showed for each article. **Impact Factor -** Impact factor of the journal by the Journal Citation Reports, year of 2019. NA – value of Impact Factor not Present. A value of 1 was assigned when the characteristic assessed was present (gray cells) and 0 when it was absent (white cells).

Table 3: Frequency of leptospirosis positive dogs weighted by the number of animals sampled for stray, sheltered and total dog population obtained 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	1.59	5	4.42	7.05	113	0.70	5	4.42	3.10
Baraitareanu, 2014	NT	NT	NT	NT	NT	77	1.09	31	40.26	43.69	77	0.48	31	40.26	19.24
Baraitareanu, 2019.	NT	NT	NT	NT	NT	19	0.27	18	94.74	25.37	19	0.12	18	94.74	11.17
Batista, 2004	130	1.44	26	20.00	28.82	NT	NT	NT	NT	NT	130	0.81	26	20.00	16.13
Belitardo, 2000	289	3.20	110	38.06	121.94	NT	NT	NT	NT	NT	289	1.79	110	38.06	68.26
Benacer, 2017	150	1.66	12	8.00	13.30	NT	NT	NT	NT	NT	150	0.93	12	8.00	7.45
Benitez, 2010.	33	0.37	7	21.21	7.76	NT	NT	NT	NT	NT	33	0.20	7	21.21	4.34
Blazius, 2005	590	6.54	62	10.51	68.73	NT	NT	NT	NT	NT	590	3.66	62	10.51	38.47
Chetta, 2014	NT	NT	NT	NT	NT	183	2.58	26	14.21	36.65	183	1.14	26	14.21	16.13
Chou, 2014	NT	NT	NT	NT	NT	720	10.15	52	7.22	73.29	720	4.47	52	7.22	32.27
Cruz-Ramero, 2013	NT	NT	NT	NT	NT	92	1.30	8	8.70	11.28	92	0.57	8	8.70	4.96
Desvars, 2013	50	0.55	23	46.00	25.50	NT	NT	NT	NT	NT	50	0.31	23	46.00	14.27
Desvars, 2012	8	0.09	7	87.50	7.76	NT	NT	NT	NT	NT	8	0.05	7	87.50	4.34
Farrington, 1982	NT	NT	NT	NT	NT	116	1.63	73	62.93	102.89	116	0.72	73	62.93	45.30
Feng, 2015	NT	NT	NT	NT	NT	720	10.15	73	10.14	102.89	720	4.47	0	0.00	0.00
Fonzar, 2012	355	3.94	41	11.55	45.45	NT	NT	NT	NT	NT	355	2.20	41	11.55	25.44
Goh, 2019	NT	NT	NT	NT	NT	193	2.72	42	21.76	59.20	193	1.20	42	21.76	26.06
Gonçalez, 2010	NT	NT	NT	NT	NT	300	4.23	28	9.33	39.46	300	1.86	28	9.33	17.37
Hafemann, 2018	NT	NT	NT	NT	NT	181	2.55	30	16.57	42.28	181	1.12	30	16.57	18.62
Ivana, 2010	NT	NT	NT	NT	NT	103	1.45	38	36.89	53.56	103	0.64	38	36.89	23.58
Jimenez-Coello, 2010	NT	NT	NT	NT	NT	224	3.16	11	4.91	15.50	224	1.39	11	4.91	6.83
Jimenez-Coello, 2008	NT	NT	NT	NT	NT	400	5.64	140	35.00	197.32	400	2.48	140	35.00	86.87
Jittapalapong, 2009	230	2.55	205	89.13	227.25	NT	NT	NT	NT	NT	230	1.43	205	89.13	127.20
Jung, 2008	NT	NT	NT	NT	NT	80	1.13	6	7.50	8.46	80	0.50	6	7.50	3.72
Khamesipour, 2014	30	0.33	12	40.00	13.30	NT	NT	NT	NT	NT	30	0.19	12	40.00	7.45
Khor, 2016.	NT	NT	NT	NT	NT	80	1.13	3	3.75	4.23	80	0.50	3	3.75	1.86
Kumar, 2009	42	0.47	4	9.52	4.43	NT	NT	NT	NT	NT	42	0.26	4	9.52	2.48
Lau, 2017	NT	NT	NT	NT	NT	96	1.35	3	3.13	4.23	96	0.60	3	3.13	1.86
Mamak, 2014.	8	0.09	2	25.00	2.22	21	0.30	0	0.00	0.00	29	0.18	2	6.90	1.24

Manić, 2014	50	0.55	4	8.00	4.43	NT	NT	NT	NT	NT	50	0.31	4	8.00	2.48
Medina 2010	NT	NT	NT	NT	NT	30	0.42	30	100.00	42.28	30	0.19	30	100.00	18.62
Meira, 2011	100	1.11	4	4.00	4.43	NT	NT	NT	NT	NT	100	0.62	4	4.00	2.48
Miotto, 2018	7	0.08	6	85.71	6.65	116	1.63	53	45.69	74.70	123	0.76	59	47.97	36.61
Myburgh, 1993	400	4.43	7	1.75	7.76	NT	NT	NT	NT	NT	400	2.48	7	1.75	4.34
Ojha, 2018	70	0.78	8	11.43	8.87	NT	NT	NT	NT	NT	70	0.43	8	11.43	4.96
Oliveira, 2012	NT	NT	NT	NT	NT	65	0.92	35	53.85	49.33	65	0.40	35	53.85	21.72
Ortega-Pacheco, 2008	350	3.88	122	34.86	135.24	NT	NT	NT	NT	NT	350	2.17	122	34.86	75.70
Paz, 2015	NT	NT	NT	NT	NT	143	2.02	22	15.38	31.01	143	0.89	22	15.38	13.65
Rivera Flores, 1999	NT	NT	NT	NT	NT	135	1.90	52	38.52	73.29	135	0.84	52	38.52	32.27
Rodríguez, 2004	197	2.18	81	41.12	89.79	NT	NT	NT	NT	NT	197	1.22	81	41.12	50.26
Ryu, 1975	1615	17.90	351	21.73	389.09	NT	NT	NT	NT	NT	1615	10.02	351	21.73	217.80
Scanziani, 2002	NT	NT	NT	NT	NT	211	2.97	71	33.65	100.07	211	1.31	71	33.65	44.06
Segovia, 2013	142	1.57	38	26.76	42.12	NT	NT	NT	NT	NT	142	0.88	38	26.76	23.58
Senthil, 2013	176	1.95	143	81.25	158.52	NT	NT	NT	NT	NT	176	1.09	143	81.25	88.73
Siam, 1973	50	0.55	6	12.00	6.65	NT	NT	NT	NT	NT	50	0.31	6	12.00	3.72
Silva, 2017	NT	NT	NT	NT	NT	425	5.99	74	17.41	104.30	425	2.64	74	17.41	45.92
Thiermann, 1980	556	6.16	187	33.63	207.29	NT	NT	NT	NT	NT	556	3.45	187	33.63	116.03
Tuemmers, 2013	400	4.43	85	21.25	94.22	NT	NT	NT	NT	NT	400	2.48	85	21.25	52.74
Vicari, 2007.	NT	NT	NT	NT	NT	64	0.90	5	7.81	7.05	64	0.40	5	7.81	3.10
Villanueva, 2018	NT	NT	NT	NT	NT	109	1.54	86	78.90	121.21	109	0.68	86	78.90	53.36
Vojinović, 2015	NT	NT	NT	NT	NT	1045	14.73	57	5.45	80.34	1045	6.48	57	5.45	35.37
Weekes, 1997	NT	NT	NT	NT	NT	78	1.10	48	61.54	67.65	78	0.48	48	61.54	29.78
Yasuda, 1980a	1415	15.69	35	2.47	38.80	NT	NT	NT	NT	NT	1415	8.78	35	2.47	21.72
Yasuda, 1980b	1428	15.83	308	21.57	341.43	NT	NT	NT	NT	NT	1428	8.86	308	21.57	191.11
Zaidi, 2018	104	1.15	5	4.81	5.54	NT	NT	NT	NT	NT	104	0.65	5	4.81	3.10
Ziehl-Quirós, 2017	46	0.51	12	26.09	13.30	NT	NT	NT	NT	NT	46	0.29	12	26.09	7.45
Zwijnenberg, 2008	NT	NT	NT	NT	NT	956	13.47	18	1.88	25.37	956	5.93	18	1.88	11.17
Total / Weighted average (%)	9021	100.00	1913	29.14	73.12	7095	100.00	1138	28.05	53.46	16116	100.00	2978	27.63	32.42

Wt (%): Weight. **Freq (%)**: frequency of positives dogs according to sampled dogs by study. **Wt freq (%)**: Weighted frequency - weight multiplied by frequency of positives dogs. NT: Not tested.

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

First author, year	**Population**	**Serovars tested**																***Leptospira* spp. serovars presented in the Vaccine**
		Cani	**Icte**	**Grip**	**Aust**	**Pomo**	**Pyro**	**Cast**	**Autu**	**Brat**	**Bata**	**Hard**	**Tara**	**Sejr**	**Sher**	**Java**	**Wolf**	
Khor, 2016	Sheltered	Neg	Neg	Neg	Neg	Neg	NT	NT	NT	NT	100%	NT	Neg	NT	NT	NT	NT	Icterohaemorrhagiae, Canicola, Pomona and Grippotyphosa
Kumar, 2009	Stray	50%	Neg	Neg	Neg	Neg	20%	NT	NT	NT	NT	Neg	20%	NT	NT	Neg	NT	Icterohaemorrhagiae and Canicola
Lau, 2017	Sheltered	Neg	Neg	Neg	33%	Neg	Neg	NT	NT	NT	33%	NT	Neg	Neg	NT	33%	NT	Icterohaemorrhagiae, Canicola, Pomona and Grippotyphosa
Miotto, 2018	Stray and Sheltered	2%	65%	7%	Neg	39%	26%	Neg	83%	Neg	Neg	Neg	Neg	2%	2%	Neg	2%	Icterohaemorrhagiae, Canicola, Autumnalis and Pomona.
Scanziani, 2002	Sheltered	14%	14%	35%	NT	4%	NT	1%	NT	55%	NT	3%	1%	NT	NT	NT	NT	Icterohaemorrhagiae and Canicola

Cani: Canicola. **Icte:** Icterohaemohrragiae. **Grip:** Grippodyphosa. **Aust:** Australis. **Pomo:** Pomona. **Pyro:** Pyrogenes. **Cast:** Castellonis. **Autu:** Autumnailis. **Brat:** Bratislava. **Bata:** Bataviae. **Hard:** Hardjo. **Tara:** Tarassovi. **Sejr:** Sejroe. **Sher:** Shermani. **Java:** Javanica. **Wolf:** Wolffi. **Neg:** Negative. **NT:** Not tested. The common serovars used in the vaccine and tested by serological tests are highlighted by grey shading.

Table 5: Significant risk factors for canine leptospirosis observed in the articles selected by this systematic review that performed this analysis.

First author, year	Population	Variable	P-value	OR	95% CI
Chou, 2014†	Sheltered 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	-	-
		Location	-	-	-
		Urban	0.008	2.23	NI
		Rural	Base category	-	-
Khamesipour, 2014	Stray dogs	*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.

Figures legends

Fig. 1: PRISMA Flow diagram of selected studies.

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

Fig. 3: Number of sampled and positives unowned (stray or / and sheltered) dogs per study selected by this systematic review.

Fig. 4: 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.

Appendix

Appendix S1: PRISMA checklist

Appendix S2: Combination of terms used at each database investigated within all the sections from papers (title, abstract and full text) in all databases, as well as the number of articles found for the search performed on September 16th, 2019 for the systematic review on Leptospira spp. infection on stray or sheltered dogs.

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

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