Occurrence of antibodies against *Leptospira* spp. in shelter dogs

Ocorrência de anticorpos contra *Leptospira* spp. em cães de abrigo

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ABSTRACT
The present study aimed to investigate the occurrence of anti-Leptospira antibodies in dogs held in a public animal shelter from the city of Barbacena, Southeastern Brazil. All samples were analyzed by the Microscopic Agglutination Test (MAT) including a panel of 24 serovars representing 21 serogroups, samples with titer ≥ 100 were defined as seroreactive. Of a total of 172 dogs investigated, 23 (13.44%) were seroreactivity with antibody titers ranging from 100 to 1600. Leptospira interrogans serovar Canicola (30.4%) (serogroup Canicola) presented the highest seroreactivity, followed by Pyrogenes (17.4%) (serogroup Pyrogenes) and Hebdomadis (13%) (serogroup Hebdomadis). The serological findings indicate that sheltered dogs are exposed to Leptospira spp. infection, with predominant of serovar Canicola historically maintained by dogs.

Key words: Canine leptospirosis, Serology, Canicola serogroup, Zoonosis, Shelter dogs.

INTRODUCTION
Leptospirosis is a zoonosis of global incidence and has been considered as a major public health problem, particularly in developing countries (ADLER & MOCTEZUMA, 2010; WORLD HEALTH ORGANIZATION, 2011; COSTA et al., 2015). This bacterial infection is caused by pathogenic species of the genus Leptospira that affects humans and many species of domestic and wild animals (BOURHY et al., 2014; SCHULLER et al., 2015a).

Pathogenic leptospires are maintained in the environment by asymptomatic renal carriers that shed the bacteria in their urine (HARTSKEERL et al., 2011; ELLIS, 2015). Most animal species may be carriers of Leptospira for long periods (BARRAGAN et al., 2017), with no disease or only mild clinical manifestations (KO et al, 2009). Exposure through water or damp soil contaminated with the urine of infected animals is the most common route of transmission to human and domestic animals (KO et al., 1999; WHO, 2003; BHARTI et al., 2003).

Rodents are the most frequent source of human infection, especially in urban areas of developing countries (BHARTI et al., 2003), where the increasing urban population, lack of basic sanitation, high rainfall, floods and high populations of maintenance hosts are important risk factors.
for infection (KO et al., 1999). However, dogs may also significantly contribute to human infection because they live in close proximity to the human population (MIOTTO et al., 2018). In this context, the evidence of seropositivity for leptospirosis in dog populations has caused public health concern (SANTANA et al., 2017).

There is no serological data about the frequency of antibodies against *Leptospira* spp. in dogs from Barbacena. Therefore, the study aimed to verify the frequency of anti-*Leptospira* antibodies in dogs held in animal shelters of Barbacena, Minas Gerais, Southeastern Brazil.

### 2 MATERIALS AND METHODS

#### 2.1 STUDY SITE AND POPULATION

The present study was conducted in the municipality of Barbacena, which is part of the Serra da Mantiqueira, a city of approximately 126,284 inhabitants (IBGE, 2010), located in the Minas Gerais State, Brazil. Barbacena has a tropical highland climate with averages annual rainfall exceeding 1,400 mm, concentrated in the spring and summer months and average temperature is 18°C.

To assess exposure to *Leptospira*, serum samples were collected from 172 apparently healthy dogs with no known history of leptospirosis vaccination. Enrollment criterion included dogs with 6 months or more admittance in the shelter, in order to avoid interference on serologic tests, since vaccination against leptospirosis is not practiced in the shelter. These dogs housed came from different regions of the city, including urban and rural areas. At the time of study, the facility conditions were considered precarious, showing unsanitary conditions, high population density and high levels of rodent infestation. In the shelter facilities, dogs were housed in groups of at least six animals, vaccinated against rabies, sterilized and most of them were prophylactically treated with anthelmintics.

All procedures involving animal manipulation in the current study were approved by the responsible of shelter through the Informed Consent Form. Approximately 5 mL of blood samples were collected from the jugular or cephalic veins. Then, the serum was centrifuged, and storage in 2mL microtubes at -20°C until the serologic tests were performed.

#### 2.2 DETECTION OF LEPTOSPIRA ANTIBODIES

Sera of sampled dogs were tested for the presence of *Leptospira* antibodies using a microscopic agglutination test (MAT) according to international recommendations (World Organization for Animal Health, 2003). The test included a panel of 24 serovars representing 21 serogroups: *L. interrogans* serogroups Australis (serovars: Australis, Bratislava); Autumnalis...
(serovar: Autumnalis); Bataviae (serovar: Bataviae); Canicola (serovar: Canicola); Hebdomadis (serovar: Hebdomadis); Icterohaemorrhagiae (serovars: Copenhageni, Icterohaemorrhagiae); Pomona (serovar: Pomona); Pyrogenes (serovar: Pyrogenes); Sejroe (serovars: Hardjo, Wolffi); Djasiman (serovar: Sentot); L. borgpetersenii serogroups Ballum (serovar: Castellonis); Javanica (serovar: Javanica); Tarassovi (serovar: Tarassovi); Celledoni (serovar: Whitcombi); L. kirschneri serogroups Autumnalis (serovar: Butembo); Grippotyphosa (serovar: Grippotyphosa); Cynopteri (serovar: Cynopteri); L. noguchi serogroup Panama (serovar: Panama); L. santarosai serogroup Shermani (serovar: Shermani); L. biflexa serogroups Andamana (serovar: Andamana); Seramanga (serovar: Patoc). Samples with titer of 100 or greater against one or more serogroups were considered as seroreactive. The titers were determined as the reciprocal of the highest serum dilutions that agglutinated at least 50% of the cells for each serovar tested. Leptosomal serogroup predominant was defined as the serogroup with the maximum titer directed against a single serovar and the remaining agglutinations were considered cross-reactions.

3 RESULTS AND DISCUSSION

Of the 172 animals investigated in the study, 23 (13.44%) were seroreactive to at least one serogroup, with titers ranging from 100 to 1600. As described in Table 1, seropositivity for MAT titer ≥100 was most frequent to serovar Canicola - serogroup Canicola (30.4%), followed by Pyrogenes - serogroup Pyrogenes (17.4%), Hebdomadis - serogroup Hebdomadis (13%), Icterohaemorrhagiae - serogroup Icterohaemorrhagiae (8.7%), Cynopteri - serogroup Cynopteri (8.7%), Wolffi - serogroup Sejroe (8.7%), Grippotyphosa - serogroup Grippotyphosa (4.3%) and Shermani - serogroup Shermani (4.3%). One sample showed equal titer against three serovars (Castellonis, Cynopteri and Grippotyphosa) and was considered undetermined.
Table 1 - Frequency of anti-Leptospiras spp. according to serovar and respective antibody titers detected in reactive sera samples of sheltered dogs in the city of Barbacena- MG, 2016

| Serogroup          | Serovar          | Antibody titre | Total (%) |
|--------------------|------------------|----------------|-----------|
| Canicola           | Canicola         | 2 1 2 2        | 7 (30.4)  |
| Pyrogenes          | Pyrogenes        | - 1 2 1 -      | 4 (17.4)  |
| Hebdomadis         | Hebdomadis       | 1 1 1 -        | 3 (13)    |
| Icterohaemorrhagiae| Icterohaemorrhagiae| - 2 - -       | 2 (8.7)   |
| Sejroe             | Wolfii           | - 1 1 -        | 2 (8.7)   |
| Cynopteri          | Cynopteri        | - 1 1 -        | 2 (8.7)   |
| Grippotyphosa      | Grippotyphosa    | - 1 - -        | 1 (4.3)   |
| Shermani           | Shermani         | - 1 - -        | 1 (4.3)   |
| *Ballum/Cynopteri/ | **Castellonis/Cynopteri/ | 1 - - -       | 1 (4.3)   |
| Grippotyphosa      | Grippotyphosa    | - - -          |           |
| Total              |                  | 4 8 3 6 2      | 23 (100)  |

*coagglutination for multiple serogroups
**coagglutination for multiple serovars

The analyses conducted in this study evidenced that shelter dogs in Barbacena are exposed to nine of the 24 pathogenic serovars tested by MAT. Despite its limitations the serology is well accepted and widely utilized to derive information about the presumptive serogroups present in a population (LEVETT, 2003; MUSSO et al., 2013), and it is indicative of the broader range of leptospires exposures by dogs as a result of a broader diversity of potential hosts that could be transmitting agent to dogs (LELU et al., 2015). However, further studies should be based on the isolation and identification of the infecting agent to better understand the real situation and dynamics of leptospirosis in this region.

In the current study, the overall frequency of Leptospira antibodies was 13.44%. This finding is consistent with review studies of canine leptospirosis in Brazil and Latin America that reported variable prevalence in dogs ranging from 7.15% to 48.2% (HAGIWARA et al., 2015) and 4.9% to 72% (PINTO et al., 2017), respectively. High frequency has been reported in shelter dogs worldwide (SCANZIANI et al., 2002; ZWIJNENBERG et al., 2008; DE PAULA et al., 2013; CHEN et al., 2014; MIOTTO et al., 2018), which is a situation that could pose a risk for transmission to other dogs, shelter workers, or adopters and a public health concern. Dogs housed in shelters are considered more susceptible to the leptospirosis infections because of shelter environmental conditions in the shelter facilities such as overcrowding, poor hygiene standards and inadequate sanitation besides the prolonged stay of a large number of dogs kept in a relatively small area that make it easier the transmission of the leptospirosis among animals (SCANZIANI et al., 2002; SPANGLER et al., 2020). Most of these conditions, such as crowding of dogs into unsanitary quarters were found in the dog shelter of this study.
The serological analysis revealed that serovar Canicola (serogroup Canicola) was the most frequency, a situation already expected, since dogs are considered maintenance hosts of this serovar (SYKES et al., 2011; MATSUI et al., 2016), and dogs shedding of the pathogen into the environment via urine and in turn increasing the risk of human contamination (GAY et al., 2014). Generally, serovar Canicola is the most frequent serovar found in infected dogs (HARKIN et al., 2003; ANDRE-FONTAINE, 2006; LELU et al., 2015); however, in areas where vaccination against serovars Canicola are common, the prevalence of seroreactivity to this serogroup in dogs is low (ANDRE-FONTAINE, 2006; SYKES et al., 2011). A recent systematic review of leptospirosis on dogs in Latin America showed the most frequent serovar predicted by the MAT was Canicola (PINTO et al., 2017). In Brazil, studies also show that in most part of country Canicola is predominant (HAGIWARA et al., 2015), and this serovar was isolated in dogs in cities of São Paulo (YASUDA et al., 1980; RODRIGUES et al., 2012) and Pelotas (BROD et al., 2005).

Pyrogenes serovar (serogroup Pyrogenes) was the second most frequent, this finding is consistent with several reports, which verified this serovar as one of the most prevalent on dog populations in divers region of Brazil (QUERINO et al., 2003; SILVA et al., 2003; BLAZIUS et al., 2005; MODOLO et al., 2006; MAGALHÃES et al., 2007; FONZAR et al., 2012; DE PAULA DREER et al., 2013; MARTINS et al., 2013) and in dogs with suspected clinical leptospirosis (AGUIAR et al., 2007; JORGE et al., 2011). Nevertheless, has not yet been detected by isolation in dogs in Brazil.

Reactions have been found for serovars Hebdomadis, (serogroup Hebdomadis) and Wolffi (serogroup Sejroe), most likely because of a cross-reaction. Historically, members of theses serogroup belonged to the larger serogroup Hebdomadis, which was divided in 1982 into three autonomous serogroups (Hebdomadis, Sejroe, and Mini serogroups) on the basis of the distribution of main antigens. However, the members of these groups still and cross-react during serological tests (KMETY, 1977; FERESU et al., 1996). Serogroup Hebdomadis has been described in humans and animals (MGODE et al., 2015), including dogs (GUERNIER et al., 2018). Infection by strains of serogroup Sejroe are reported in cattle worldwide (ELLIS, 2015; PINTO et al., 2016) and other study have reported disease in association with serogroups Sejroe in dogs (RÜHL-FEHLERT et al., 2000). In Asia, dogs were previously identified carrying L. wolffii, although the pathogenicity of this serovar for dogs has not been proven (ZAKERI et al., 2010). It is noteworthy that some shelter dogs may be from rural areas since the shelter collects stray dogs from all regions of the municipality, including dogs from the rural area. These findings may be indicated that bovine specie is involved in the epidemiology of canine leptospirosis in the study area.
Other serovars were detected at lower frequencies, such as Icterohaemorrhagiae (serogroup Icterohaemorrhagiae) and Grippotyphosa (serovar: Grippotyphosa), which were mainly associated with rodent species that are usually found in urban areas and wild mammals, respectively. Dogs are accidental hosts of the serovar Icterohaemorrhagiae (SCHULLER et al. 2015b) and the importance of this serogroup as a cause of severe illness in humans should be highlighted.

It is noteworthy that serological data from dogs should be interpreted with caution because vaccination of dogs could be responsible for the serological findings against serovars present in vaccines. It is known that titers of antibodies induced by vaccination are low (BARR et al., 2005; GONÇALES et al., 2013) and only maintained for three-four months in most cases (BOLIN, 1996; KLAASEN et al., 2003). A small proportion of vaccinated dogs may still develop antibodies detected up to six months (ARDUINO et al., 2009). However, all the dogs had been housed in the shelter at least six months prior to the study and vaccination against leptospirosis is not practiced in the shelter due to economic restraints.

Additionally, high titers (≥800) were found for the serovars Canicola, Pyrogenes, Hebdomadis, Woffii e Cynopteri. It is known that titers of 800 or above in dogs from endemic regions are indicative of acute infection (ZWIJNENBERG et al., 2008; FRAUNE et al., 2013), although all the sheltered dogs were reported to be healthy for the leptospirosis. These findings tend to suggest that leptospirosis infection, especially for the serovar Canicola, is important in the study population and that general signs of leptospirosis are difficult to notice in a shelter setting.

4 CONCLUSIONS

Serological evidence of canine leptospirosis in Barbacena showed that sheltered dogs are exposed to Leptospira spp. infection, with predominance of serovar admittedly maintained by dogs. The results suggest the implementation of immunization programmes, using vaccines containing serovar Canicola in order to mitigate the impact of this disease on the canine population and in other species, including humans. Further studies are warranted for investigation whether the infected dogs are carriers of leptospires and therefore contribute to environmental contamination.

ETHICS STATEMENT

All procedures involving animals were approved by the Ethic Committee on Animal Use of the University Santo Amaro (CEUA/UNISA n° 08/2015).

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