Short Communication

Serological and molecular detection of Leptospira spp in dogs

Giulia Soares Latosinski[1], Felipe Fornazari[1], Selene Daniela Babboni[1], Karen Caffaro[1], Antonio Carlos Paes[1] and Helio Langoni[1]

[1]. Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, SP, Brasil.

Abstract

Introduction: This study aimed to detect anti-Leptospira spp antibodies and Leptospira DNA in domestic dogs. Methods: Blood and urine from 106 dogs were evaluated by microscopic agglutination test (MAT) and polymerase chain reaction (PCR), respectively. Results: Six (5.7%) and one (1%) animals were positive by MAT and PCR, respectively. Conclusions: These results show a low prevalence of infection by Leptospira spp. The absence of positive results for the Icterohaemorrhagiae serogroup indicates the small relevance of these dogs as sources of human leptospirosis.

Keywords: Leptospirosis. PCR. Microscopic. Agglutination test.

Leptospirosis is a zoonotic disease that affects humans and domestic and wild animals through direct or indirect contact with the urine of infected hosts, mainly rodents. Environmental risk factors contribute to the endemic character of the disease, especially in developing countries. In Brazil, poor sanitary conditions are common, including untreated sewage and garbage accumulation, which predisposes the proliferation of rodents and thus may expose humans, dogs, and other animals to leptospirosis.

Leptospirosis is considered an emerging disease due to its increased incidence among populations such as domestic dogs in some regions of the world. This change may be associated with climate change, which favors the increased survival of leptospires in the environment. Tropical climate, standing water, poor sanitation, and proximity to animal reservoirs intensify the epidemic character of leptospirosis in developing countries. Besides rodents, dogs can also play an important role in the epidemiology, acting as accidental or maintenance hosts. Dogs can also be sentinels for several diseases, assisting in pathogen detection in a particular area.

The gold-standard serological test for leptospirosis is the modified agglutination test (MAT), which has a specificity and can be used to identify the infecting serogroup of the bacterium, thus helping to detect the probable animal source of infection. Polymerase chain reaction (PCR) is another important diagnostic test, which detects leptospiral deoxyribonucleic acid (DNA) in several types of samples, including blood, urine, semen, and organs. Compared to bacterial culture, PCR is faster, more specific, and sensitive.

The present study aimed to evaluate the role of dogs in the epidemiology of human leptospirosis in Botucatu county, São Paulo, Brazil, through the detection of anti-Leptospira antibodies and leptospiral DNA in dogs. According to the serogroups identified by MAT and the frequency of animals shedding leptospiral DNA, we can propose if dogs may be involved in the epidemiology of human leptospirosis.

Blood and urine samples were collected from 106 asymptomatic dogs in Botucatu County, São Paulo State, Brazil, which has an estimated dog population of 26,721 animals and a prevalence of anti-Leptospira antibodies ranging from 15 to 20% in asymptomatic dogs, according to previous investigations. The study was conducted between October 2014 and June 2015. Samples were collected during the municipal vaccination campaign against rabies in the Municipal Kennel of Botucatu and during medical care at the Veterinary Hospital from the School of Veterinary Medicine and Animal Science [Faculdade de Medicina Veterinária e Zootecnia (FMVZ)], São Paulo State University, Botucatu [Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP)]. All samples were collected with consent of the dog’s owner. Phosphate buffered saline solution (PBS) pH 7.6 was added to urine in a 1:1 proportion and the tubes were frozen at -20°C. Most of the dogs included in the study were stray animals taken to the municipal kennel for castration. Thus, epidemiological data were lacking.

Detection of antibodies was performed using the MAT. A collection of 12 antigens maintained at 28°C in Ellinghausen-McCullough-Johnson-Harris media (EMJH), was used: Australis,
Table 1: Results of the MAT in sera samples of dogs from Botucatu County, São Paulo State, Brazil.

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Titers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Canicola</td>
<td>-</td>
</tr>
<tr>
<td>Autumnalis</td>
<td>-</td>
</tr>
<tr>
<td>Grippotyphosa</td>
<td>-</td>
</tr>
<tr>
<td>Autumnalis + Canicola</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
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</table>

MAT: microscopic agglutination test.
lower prevalence. None of the dogs in our study were positive for the Icterohaemorrhagiae serogroup, which is the most important in public health. Therefore, it is unlikely that these animals play a major role in the epidemiology of human leptospirosis in the region of Botucatu. A recent study reported a high prevalence of dogs positive for serogroup Icterohaemorrhagiae in PCR of urine sample\(^{14}\). This study was conducted in a leptospirosis-endemic area, which probably explains the difference in relation to our results since Botucatu is a non-endemic city. Between 2010 and 2015, only nine cases of human leptospirosis were notified according to Brazil’s Information System for Notifiable Diseases. Researchers have debated if dogs are really relevant in the transmission of leptospirosis to humans\(^{15}\) and, until now, there has been no consistent data on this fact.

The antibody titers ranged from 200 to 1,600 and three animals had titers equal to or higher than 800. The cut-off of 800 is considered indicative of clinical disease in humans. However, there are not currently any specific standard criteria for dogs. All dogs in this study were apparently healthy, indicating that high titers are not always associated with disease symptoms. This finding is corroborated by our personal experience as well as by the literature\(^{16}\). Thus, it is important to associate laboratory finding is corroborated by our personal experience as well as by the literature\(^{16}\). Thus, it is important to associate laboratory
diagnosis with clinical history and epidemiology so MAT results can be interpreted properly.

PCR allowed us to assess the potential of dogs as carriers of leptospires. Few studies using PCR in dogs have been performed, especially in Brazil. Our results indicated a low frequency of dogs carrying leptospires in the urine. Bacterial shedding is intermittent; thus, sampling animals more than once could indicate a higher prevalence. However, in this case, we believe that the number of dogs positive by PCR would still be low, corroborating the low frequency of positive animals by MAT. It is also possible that the PCR results were associated with small concentrations of leptospires in urine, which can be below the PCR detection threshold when animals are chronically infected by adapted serovars.

The dog positive by PCR was negative by MAT, a result that can be explained by the pathogenesis of leptospirosis. Antibodies can be detected 10 to 14 days after infection, with high levels between 21 and 42 days that can be maintained for six weeks. A gradual reduction occurs until titers are low (or undetectable). Leptospiuria starts 14 days after infection, is intermittent, and can last for just a few days or more than two years\(^{3}\). In addition, the Leptospira spp detected by PCR could belong to a serogroup that was not included in the MAT. Disagreement between MAT and PCR is common and has been observed in many studies regardless of animal species\(^{5,14}\).

One of the limitations of this study was the low sample size as it was not representative of the dog population in Botucatu. We did not sample more animals because this preliminary investigation focused mainly on the role of dogs as carriers of leptospires. In addition, the narrow confidence interval of the PCR results (0.2-2.7) indicates the accuracy of these data.

In conclusion, these results contribute to the understanding of leptospirosis epidemiology in the study region. The investigated dogs had a low prevalence of infection by Leptospira spp, with a higher positivity for Canicola serogroup. The absence of positivity for the Icterohaemorrhagiae serogroup suggests that these dogs are not involved in the epidemiology of human leptospirosis. This hypothesis is reinforced by the low frequency of dogs shedding leptospires. However, the detection of just one animal positive by PCR could have significant implications for environmental contamination depending on the pathogenicity of the leptospires, bacterial load in urine, its survival in the environment, and the shedding period. More studies are needed to address these questions.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES


