

Spatial analysis of leptospirosis and toxoplasmosis seroprevalence in the canine population in an area of socioeconomic and environmental vulnerability

[Análise espacial da soroprevalência de leptospirose e toxoplasmose na população canina em área de vulnerabilidade socioeconômica e ambiental]

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ABSTRACT

Leptospirosis and toxoplasmosis are zoonoses of occupational character that are spread worldwide, mainly in tropical and socioeconomically vulnerable areas. Both diseases have vertebrate hosts, which spread the infectious agent to the environment and are potentially infectious to humans and can progress to fatal cases. The present study reinforces the importance of One Health for the control and prophylaxis of leptospirosis and toxoplasmosis using sentinel animals in areas of socioeconomic vulnerability based on a serological study. A total of 336 blood samples were obtained from the canine population of the urban district, which has high rates of poverty and sanitation. The results were analyzed based on seroreactivity animals and georeferencing by kernel spatial mapping. A total of 43.3% (52/120) of the animals were seroreagents for anti-*Leptospira* spp., with a predominance of *Canicola* (35/52 = 67.3%) and *Pyrogenes* (5/52 = 9.6%), while 52.97% (178/336) of the dogs were seroreactive for anti-*Toxoplasma gondii*. The presence of seroreagent dogs for both zoonosis alert to the inadequate hygienic-sanitary conditions of the studied region, and highlight the importance of a survey of the social, economic and environmental structure to identify factors related to the links in the epidemiological chain of pathogen transmission and necessary corrective actions.

Keywords: Leptospirosis, *Toxoplasma gondii*, Zoonoses, single health, vulnerable population.

RESUMO

Leptospirose e toxoplasmose são zoonoses de caráter ocupacional disseminadas mundialmente, principalmente em áreas tropicais e socioeconomicamente vulneráveis. Ambas as doenças têm hospedeiros vertebrados, que espalham o agente infeccioso para o meio ambiente e são potencialmente infecciosos para o homem, as quais podem evoluir para casos fatais. O presente estudo reforça a importância da saúde única para o controle e a profilaxia da leptospirose e da toxoplasmose, mediante a utilização de animais sentinela em áreas de vulnerabilidade socioeconômica, com base em estudo sorológico. Um total de 336 amostras de sangue foi obtido da população canina de um distrito urbano que apresenta altos índices de pobreza e ausência de saneamento básico. Os resultados foram analisados com base em animais sororreagentes e georreferenciados por mapeamento espacial de Kernel. Um total de 43,3% (52/120) dos animais foi sororreagente para anti-*Leptospira* spp., com predominância de *Canicola* (35/52 = 67,3%) e *Pirogenes* (5/52 = 9,6%), enquanto 52,97% (178/336) dos cães foram sororreagentes para anti-*Toxoplasma gondii*. A presença de cães sororreagentes para ambas as doenças alerta para as condições higiênico-sanitárias inadequadas da região estudada e evidencia a importância de um levantamento da estrutura social, econômica e ambiental para identificar fatores relacionados aos elos da cadeia epidemiológica de transmissão do patógeno e ações corretivas necessárias.

palavras-chave: leptospirose, *Toxoplasma gondii*, zoonoses, saúde única, população vulnerável

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INTRODUCTION

Toxoplasmosis and leptospirosis are notifiable zoonoses and require numerous health surveillance strategies to control and eradicate new cases, as they can progress to fatal cases. The prevalence and incidence of these zoonoses, in turn, are associated with places of poverty, in which the lack of basic sanitation and health education contribute to the emergence of new cases (Fung *et al.*, 2014). Epidemiological surveillance of zoonoses is an important tool for the control and prophylaxis of diseases at a global, national, regional, or municipal level. Active and passive defense strategies are widely implemented in Brazil. However, in areas of critical poverty, with deficiencies in the availability of electricity and means of transport, such populations are exposed to different zoonoses (Primeiro..., 2012).

In places of extreme poverty, in addition to the difficulties of medical care, veterinary medical care also becomes precarious for sick animals, sometimes with zoonoses, facilitating the spread of pathogens (Fung *et al.*, 2014).

Leptospirosis is an infectious disease that affects humans and animals and is caused by the bacterium *Leptospira interrogans*, which has a worldwide distribution and is endemic in Brazil. It is estimated that, on average, 3,800 human cases are reported each year. It is present in regions of greater socioenvironmental and economic vulnerability, particularly in regions with high rainfall, which is considered a seasonal disease (Chaiblich *et al.*, 2017; Marteli *et al.*, 2020). The vole (*Rattus norvegicus*) is one of the main reservoirs of the pathogen. The elimination of the bacteria occurs through urine, mainly infecting humans and accidental and terminal hosts of the cycle, through contact with contaminated water. Other mammals, including companion animals such as dogs and cats, can develop the clinical form of the disease or become sources of infection due to the elimination of the pathogen in the urine and infection of other susceptible animals, in addition to widespread contamination of the environment through fomites, water, pastures and soil (Greene, 2012; Silva and Silva, 2016).

Specific preventive measures, such as vaccination of animals and sand cattle have been

indicated for the control of human cases (Primeiro..., 2012). The diagnosis in case of suspicion of laboratory disease is a microscopic seroagglutination with live antigens (SAM), in which the serum is tested for several serovars, representatives of the main serogroups, *Leptospira* spp. Serum levels of healthy individuals were quantified (Brasil, 2014).

Toxoplasmosis is another endemic zoonosis in Brazil that is activated by the protozoan *Toxoplasma gondii*. Commonly, *T. gondii* infections are opportunistic. It is estimated that the disease affects approximately 500 million humans worldwide (Greene, 2012; Passos *et al.*, 2018). Infection by *T. gondii* varies globally in different lifestyles and diets (Coelho *et al.*, 2003; Pappas *et al.*, 2009; Parasites..., 2018). In dogs, the disease presents clinical signs such as enteric, ocular and/or neuromuscular. Felids are the definitive hosts of this protozoan, eliminating the cyst through feces, representing sources of contamination for vegetables, fountains, water and pasture soil (Greene, 2012; Passos *et al.*, 2018; Bresciani *et al.*, 2008).

Dogs do not shed *T. gondii* oocysts in feces (Lindsay *et al.*, 1997). However, they are considered risk hosts for human infection, as they can act as mechanical conveyors, carrying the sporulated oocyst in the fur, particularly those in which there is cohabitation with felines, due to the common habit of xenophilia. Furthermore, the diagnosis of toxoplasmosis in dogs can serve as epidemiological surveillance of the disease for humans (Lindsay *et al.* 1997; Camossi *et al.*, 2008; Cunha *et al.*, 2016).

The ingestion of sporulated oocysts by dogs as a result of the feline coprophagy habitual turns the dog into a mechanical host, as they can eliminate oocysts as a still infective form by samples contaminating the environment (Lindsay *et al.*, 1997); Cunha *et al.*, 2016; Leal and Coelho, 2014).

Diagnosis in dogs is routinely performed by the indirect immunofluorescence reaction (IFAT) technique and quantification of anti-*T. gondii*. Although the RIFI does not determine the cutoff point for animals considered to be seropositive, the results are associated with clinical-epidemiological findings (Brasil, 2014). Greene (2012) stated that, in the serological diagnosis of

Spatial analysis...

dogs, high titers of immunoglobulins with a fourfold increase in titer or more are indicative of acute disease, while low titers without the presence of clinical signs may indicate previous infection (Silva and Silva, 2016).

Studies carried out in Brazil have observed a wide variation in the seroprevalence of canine toxoplasmosis (Coelho *et al.*, 2003; Pappas *et al.*, 2009). It is believed that socioeconomic factors, such as poverty and different cultural and life habits, in addition to the supply of raw meat and hunting, influence the prevalence and occurrence of the disease (Arruda *et al.*, 2021; Moura *et al.*, 2007). Cañón-Franco *et al.* (2004) and Arruda *et al.* (2021) demonstrated by serological techniques that mixed-breed dogs are more predisposed to *T. gondii* infections, probably due to free access to animal carcasses, untreated water sources, feline feces, and other factors that are involved in the transmission of the agent (Arruda *et al.*, 2021; Cañón -Franco *et al.*, 2004; Brescianni *et al.*, 2016).

Serological surveys performed with dog sera provide epidemiological indications for human exposure to the pathogen. They make it possible to understand the spread of this zoonosis in various habitats, since dogs cohabit with humans, sometimes maintaining close contact with the animals, and in several situations, there are intimate effects (Camossi *et al.*, 2008; Cunha *et al.*, 2016; Leal and Coelho, 2014).

In a study on demographic data and health indicators in the area covered by primary health care units conducted between 2000 and 2004 in the city of Botucatu “22°S, 48°W”, a comparative analysis was carried out between the city of Botucatu and its coverage areas, including the district of Rubião Junior (Carancina and Almeida, 2005). It was observed that demographic variations in the Rubião Junior region were closely related to health status and access to healthcare, including age pyramid, total deaths, birth rate, mortality coefficients, and mortality from underlying causes. Deaths were evaluated according to age group, and Rubião Junior was among the regions with the highest percentage of mortality among children under 1 year of age, surpassing the city of Botucatu. High infant mortality rates are related to nutritional scarcity, infections, lack of primary

prevention/vaccination, insufficient health care, and inadequate sanitation.

Considering domestic animals as sentinels for the surveillance of infectious diseases, such as leptospirosis and toxoplasmosis, and the influence of socioeconomic vulnerability on the spread of pathogens, seropositivity for leptospirosis and toxoplasmosis was observed in 438 dogs belonging to the district of Rubião Junior in the municipality of Botucatu, Sao Paulo, Brazil.

MATERIALS AND METHODS

The study was carried out by following Brazilian legislation on the use of animals in research and teaching (Law n° 11.794/2008, of Decree n° 6.899/2009) and approved by the National Council for the Control of Animal Experimentation (CONCEA) of the State University of São Paulo (UNESP), Brazil, under protocol 36/2016.

Samples of 336 dogs, with or without a defined breed, of both sexes and different ages without presenting any clinical signs belonging to the urban district of Rubião Júnior, municipality of Botucatu, SP, where there is marked socioeconomic vulnerability, were studied. According to IBGE (Cidades..., 2010), the estimated population of Botucatu in 2021 was 149,718 habitants and 27,735 dogs in 2018. There was no limit to the sample number; the samples were obtained for convenience, according to the authorization of the tutor during the vaccination campaign against distemper, in 2014 and after completing a questionnaire about the animal's data (gender, age, breed, domiciled or not, community dog, eating habits and contacts with other animals) the blood collection was then done. Aggressive animals, which made the collection impossible or presented alterations of clinical parameters during the vaccination campaign were excluded. The project was supported by the Dean of Extension at UNESP, the Faculty of Veterinary Medicine and Animal Science - FMVZ at UNESP, and the Health Department of the Municipality of Botucatu, SP.

The target audience of the campaign was low-income tutors, and the location was chosen as the study area because it is in the peripheral region

of the municipality and due to its proximity to the FMVZ of UNESP/Botucatu, SP.

In parallel with the multiple vaccination of the animals, lectures were also held at the Basic Health Units for clarification on the campaign, in addition to information on diseases and carrying out the deworming of the animals.

The animals were clinically evaluated for rectal temperature, mucosal color, and heart and respiratory rate before sample collection, and those that showed changes in normal parameters were excluded. Blood collection was performed by jugular or cephalic vein puncture. After previous asepsis, with the AID of sterile needles (30 x 8 mm) and 5 mL syringes, the samples were collected and placed in sterile dry tubes without anticoagulant. After retraction of the clot, the samples were centrifuged for ten minutes at 2000 rpm, obtaining clear sera, which were kept at -20°C in aliquots until the serological tests were performed. The number of aliquots from the same animal was a limiting factor due to the volume of the collection, resulting in differences in samples for performing serologies.

The samples were submitted to Microscopic Seroagglutination (MAT), considered standard by the Brazilian Ministry of Health (Brasil, 2014).

Briefly, serum samples were initially diluted 1:50 in sterile PBS, pH 7.2, to assess the initial screening titer (after antigen inclusion) of 100. Serum was considered reactive when 50% agglutination occurred or more of the leptospire about to with concerning the control (Bolin, 1996). A panel of 22 serovar antigens was used, and the serovar most likely to be infected was considered the one with the highest agglutinating titer (Modolo et al., 2006; Vasconcellos et al., 1997). In the event of the highest titer reacting to more than one serovar, the sample was characterized as co-agglutinated (Bolin, 1996).

Indirect immunofluorescence for *T. gondii* was performed according to Camargo (1974) using slides previously sensitized with tachyzoites and

sera at dilutions from 1:16 to 1:256. The 16 IU titer was considered the cutoff point for positive results.

Descriptive statistical analysis was performed to present the relative and absolute frequencies of the IFI results. Chi-square or Fisher's exact test (PROC FREQ, SAS Institute, Cary, NC, USA) was used to investigate the association between epidemiological variables and serological results for both diagnoses (positive and negative). The analysis was performed by adopting a significance level of $p < 0.05$.

To estimate the density and intensity, through heat points (hot spots), of the seroreagents animals, the kernel density technique was used (Chaiblich et al., 2017; Almeida Junior, 2017; Minas Gerais, 2019). The maps were generated by ArcMap 10.5 - ESRI software, using the "Kernel Density" module with the parameters: area unit: square meters, output values: Density and method: Planar. The coordinate system used was GCS_SIRGAS_2000 and planimetric datum D_SIRGAS_2000, with spheroid GRS_1980.

RESULTS

The presence of reactive dogs for anti-*Leptospira* spp was observed in 43.33% (52/120) of the sampled animals. The serovar Canicola was the most frequent (35/52 = 67.30%), followed by Pyrogenes (5/52 = 9.62%) and Copenhagen (3/52 = 5.77%). The distribution of the titles of the dogs sampled is summarized in Table 1, with identification of the serovars performed and coagglutinations (Table 1).

No significant differences were observed associated with the risk factors studied by the epidemiological survey, including contact with cats and type of diet (commercial food, homemade or mixed food).

A high concentration of reagents animals for anti-*Leptospira* spp and anti-*T. gondii* was observed, as evidenced by the gradation of colors (from blue to red). The shade closest to red is the area with a predominance of seroreagents animals (Figures 2 and 3).

Spatial analysis...

Table 1. Distribution of maximum serovar titers and anti-*Leptospira* coagglutinations in the microscopic seroagglutination test (SAM) in 53 reagents sera from dogs sampled in the distemper vaccination campaign. Rubião Júnior District, Botucatu, SP (2013 to 2014)

Serovar	Titer							Total N (%)
	200	400	800	1600	3200	6400	12800	
Canicola	10	5	8	8	1	2	1	35 (67.30)
Pyrogenes	4	1	-	-	-	-	-	5 (9.62)
Copenhageni	2	-	1	-	-	-	-	3 (5.77)
Butembo	1	1	-	-	-	-	-	2 (3.86)
Canicola/ Pyrogenes	2	1	-	-	-	-	-	3 (5.77)
Copenhageni/ Pyrogenes	1	-	-	-	-	-	-	1 (1.92)
Icterohaemorrhagiae/ Pyrogenes	1	-	-	-	-	-	-	1 (1.92)
Pomona/ Grippotyphosa	-	-	1	-	-	-	-	1 (1.92)
Canicola/ Pomona/ Grippotyphosa	-	-	1	-	-	-	-	1 (1.92)
Total	21	8	11	8	1	2	1	52 (100)

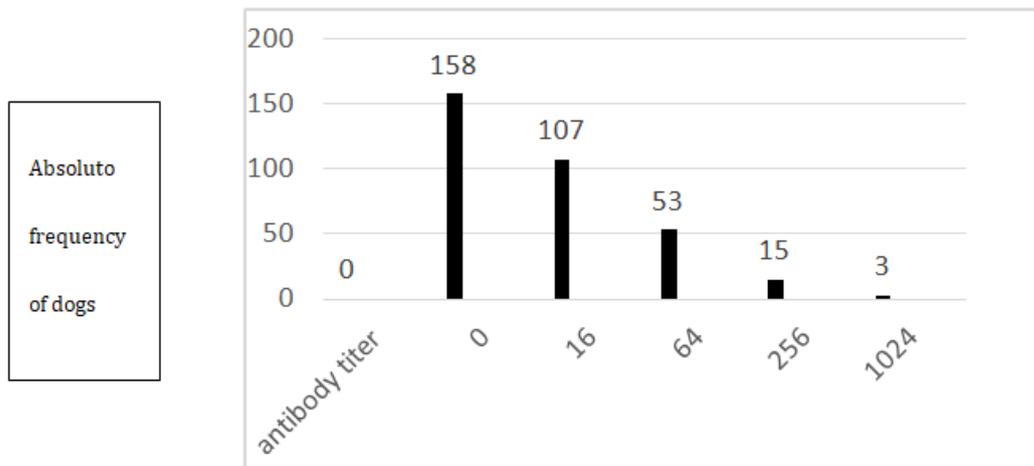


Figure 1. Distribution of anti-*T. gondii* antibody titers by the Indirect Immunofluorescence Reaction Technique in dogs from the Rubião Júnior District, Botucatu, SP (2013-2014).



Figure 2. Kernel spatialization in seroreagents dogs for anti-*Leptospira* spp from the District of Rubião Júnior, SP (2013-2014).

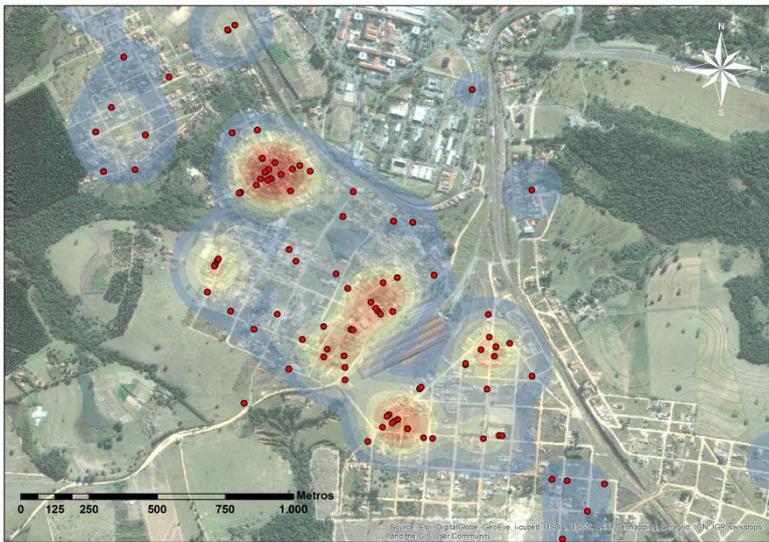


Figure 3. Kernel spatialization in seroreagents dogs for anti-*T. gondii* from the District of Rubião Júnior, SP (2013-2014).

DISCUSSION

Studies that investigate the seroprevalence of pathogens with zoonotic potential in the canine population are of vital importance in the epidemiological surveillance of diseases for human populations. This monitoring is relevant due to the close relationship between humans and dogs today, which makes interspecies coexistence a worrisome factor for the sanitary control of diseases (Arruda *et al.*, 2021; Canon-Franco *et al.*, 2004).

The SAM test requires caution in its interpretation to obtain a definitive diagnosis, as it is an indirect diagnostic technique (Tizard, 1998; Ribeiro *et al.*, 2003). A single exam with a titer greater than or equal to 100 may indicate residual antibodies from a previous infection, a response to a subclinical infection, or recent vaccination, thus recommending paired serology.

The serovar Canicola is pointed out as the variant most associated with dogs, its main reservoir³¹. In Brazil, serological findings in dogs indicated the seroprevalence of different serovars, such as Icterohaemorrhagiae, Copenhageni, Canicola, Pyrogenes, among others, varying in frequency between different researchers and regions (Coiro *et al.*, 2011).

The seroprevalence for leptospirosis investigated in the municipality of Pardinho “23°S, 48°W” (a region neighboring the municipality covered by the present study) identified 3.87% (17/181) seroreagents dogs, with a predominance of the serovar Canicola (71, 43%), followed by Icterohaemorrhagiae and Copenhageni, of which the latter are serovars related to more severe clinical conditions in humans (Bresciani *et al.*, 2008; Cañón -Franco *et al.*, 2004). Different studies have reported the Canicola serovar as the most frequent in the canine population, including the municipality of Botucatu, ranging from 10.2% to 40.3% (Modolo *et al.*, 2006; Langoni *et al.*, 2015). The present study also observed the predominance of serovar Canicola (67.30%), reinforcing the wide circulation of this serovar in the region. The zoonotic potential of dogs infected with this serovar should still be highlighted, as they can eliminate the agent in the environment for a long period (Furtado *et al.*, 1997; Hagiwara *et al.*, 2004).

The serovar Pyrogenes is considered an accidental pathogen for dogs (Jouglard and Brod, 2000). In a study carried out with stray dogs in Santa Catarina, results similar to those obtained in the present study were observed, with a prevalence of 18% for serovar Pyrogenes, followed by 13.8% for serovar Canicola (Blazius *et al.*, 2005). In the Botucatu region, serovar

Spatial analysis...

Pyrogenes were also frequent, with 34.5% (41/119). In contrast, in a study by Langoni *et al.* (2015) with dog sera from the Zoonosis Control Center, the serovar Pyrogenes was not identified, reinforcing the geographic variability of dogs seroreagents for leptospirosis (Langoni *et al.*, 2015; Greene *et al.*, 2006).

In this context, a case of conjunctival transmission of leptospirosis between a dog and its caregiver was reported, warning of the need for actions aimed at health education for the general population, such as zoonoses and responsible ownership of companion animals. Consideration should be given to the asymptomatic chronicity of dogs with leptospirosis, the wide ease of movement and the reservoirs of maintenance of the agent in the environment, which make it difficult to cope with leptospirosis in dogs (Polachini and Fujimori, 2015).

The high frequency of leptospirosis cases in regions with poor sanitary infrastructure, with a predominance of the community living in poverty and difficulties in accessing basic health services, places leptospirosis in the context of neglected tropical diseases (NTDs) and related to populations in situations of socioeconomic and socioenvironmental vulnerability. A spatial study of risks to leptospirosis carried out in the city of Rio de Janeiro revealed three neighborhoods with the highest incidence rate of leptospirosis, in which inadequacies were observed in the sanitary services of garbage collection, treatment of sewage, water supply, and adequate urban infrastructure for rainwater drainage, reinforcing the prevalence of the disease in regions with deficiencies in public health services and basic sanitation (Chaiblich *et al.*, 2017; Bresciani *et al.*, 2008).

Leptospirosis is an endemic infectious disease in Brazil. Between 2007 and 2017, the annual average was 3,846 cases in humans, with a high prevalence in the South and North regions (Marteli *et al.*, 2020). In addition to socioeconomic vulnerability, the environmental characteristics of the region can also influence the prevalence of leptospirosis in the local community (Chaiblich *et al.*, 2017; Bresciani *et al.*, 2008). Based on geoprocessing analyses, the natural profile of regions was related to the incidence, considering the frequency of

hydrometeorological phenomena, population density, relief, and variations in climatic conditions, finding a close relationship between leptospirosis and high rainfall. Additionally, in places whose relief may predispose the occurrence of flooding, there is a tendency to increase the number of cases. The studies that relate the prevalence of leptospirosis and the natural patterns intrinsic to a particular location are accompanied by the analysis of the social profile of the same since the presence of structural inadequacies can result in the intensification of the events that favor the dissemination of the pathogen of this zoonosis^{3,4,12}. Furthermore, areas with socioeconomic vulnerability tend to favor the proliferation of natural reservoirs of *Leptospira* spp., especially the vole (*Rattus norvegicus*), in rural areas (Machado *et al.*, 2017).

The seroprevalence of leptospirosis in 43.33% of the animals sampled in the present study indicates the circulation of the etiologic agent in the canine population of the urban district of Rubião Júnior. The deficient sanitary conditions and precarious urban infrastructures, associated with a predominantly low-income population and without knowledge of the relevance of this zoonosis, are risk factors that place the studied region in a situation of socio-economic and environmental vulnerability, a fact that contributes to the spread of leptospirosis in the canine population (Carandina and Almeida, 2005). In this scenario, it was possible to analyze possible risk factors for leptospirosis, including contact between dogs participating in the campaign with other dogs and the presence of rodents. Among the animals seroreagents for *Leptospira* spp., 38.27% had contact with other dogs, and 33.33% had a history of the presence of rodents in the environment. Despite the lack of statistical significance, the presence of these factors indicates the low sanitary condition of the region.

Among the seroreagents samples, the serovars Copenhageni and Icterohaemorrhagiae, which are related to severe clinical manifestations in humans, were identified (Brasil, 2019). Considering the potentially lethal evolution of leptospirosis, the presence of these serovars is a concern for local authorities, since the circulation of the pathogen signals a greater occurrence of cases of the disease.

Coelho *et al.* (2003) investigated environmental risk factors related to canine toxoplasmosis in a district of Botucatu marked by socioeconomic and environmental vulnerability. It was observed that 56% of the animals sampled had anti-*T. gondii* antibodies, of which, among the seroreagents, 57.14% resulted in titer 16. In the same study, the group of dogs with access to the street and fed raw meat showed a higher frequency of seroreagents. Such findings highlight the importance of the environment in the epidemiology of the disease, permanence, and transmission of the etiologic agent, a fact that would partially justify the difficulties in controlling *T. gondii* infections in areas with precarious environmental conditions (Coelho *et al.*, 2003). Our study was carried out almost eleven years later and found a similar prevalence (52.97%), indicating lack of investment and actions by the public authorities.

A study of the seroprevalence of *T. gondii* in dogs in the state of Rio de Janeiro found 46.08% (300/651) seroreagents animals (Cunha *et al.*, 2016). Similarly, the seroepidemiological analysis of toxoplasmosis carried out in Jataizinho “23°S, 50°W” PR, CO, with 766 dogs, identified 52.35% seroreagents animals, associating the reagents dogs with free access to street and the presence of stray dogs, reinforcing the prevalence of disease in vulnerable areas from economic, social, and environmental aspects (Lee *et al.*, 2015).

Studies carried out in Brazil, in different states and cities, alert to the negligence or underdiagnosis of canine toxoplasmosis, with predominance in areas of socioeconomic, social and environmental vulnerability. The disease in dogs is linked to precarious sanitary conditions and environmental aspects, which are risk factors and are common in places marked by intense social inequality and low economic conditions. Likewise, the tropical climate, the presence of stray animals and wild reservoirs and the close relationship between humans and dogs are factors that contribute to the permanence and spread of the pathogen (Souza and Belfort, 2014).

The presence of seroreagents dogs for *Leptospira* spp. and *T. gondii* in the sampled animals alert to the inadequate hygienic-sanitary conditions of the studied region, indicating the need to adopt

actions to solve the difficulties in this area of socioeconomic and environmental vulnerability, including a survey of the social, economic and environmental structure to identify factors related to the links in the epidemiological chain of pathogen transmission. The work of Carandina and Almeida (2005) highlights the demographic variants of the Rubião Junior region with a close relationship to sanitary conditions and access to healthcare, including age pyramid, total deaths, birth rate, mortality coefficients, and mortality from underlying causes.

CONCLUSION

The present study observed a large number of seroreagents dogs for anti-*Leptospira* spp and anti-*T. gondii*, which represent diseases of known zoonotic impact in a region characterized by high social vulnerability, emphasizing the dog as sentinel animals for epidemiological surveillance studies of the diseases, the interrelationship of leptospirosis and toxoplasmosis with aspects of socioeconomic and environmental vulnerability and the importance of the One Health concept in the adoption of control/prophylaxis actions in the fight against these diseases.

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