

Article/Artigo

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

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ABSTRACT

Introduction: Leptospirosis is a zoonotic disease, the primary hosts of which are wild, synanthropic, and household animals. Humans behave as terminal and accidental hosts. The prevalence of leptospirosis depends on carrier animals that disseminate the agent, on the environmental survival of this agent, and on the contact of susceptible individuals. Each serovar has one or more hosts with different adaptation levels. The focuses of leptospirosis are infected, sick, and asymptomatic animals, which are considered to be sources of environmental infection. This study aimed to determine the risk areas for leptospiral infection in stray dogs and patients diagnosed with leptospirosis from 2006 to 2008 in Maringá, State of Paraná, Brazil. Methods: Three hundred and thirty-five stray dogs and 25 patients were studied. Serum from both animals and patients was examined by the microscopic serum agglutination test to study anti-leptospiral antibodies. To determine the risk areas and the spatial distribution of the disease, thematic maps were designed. Results: Forty-one (12.2%) dogs positive for one or more leptospire serovars were observed, the most frequent serovars being Pyrogenes (43.9%), Canícola (21.9%), and Copennhageni (19.5%). Among the humans, 2 (8%) were positive for serovars Pyrogenes and Hardjo Prajitno and for Pyrogenes and Cynopteri. Conclusions: Spatial analysis showed that the risk for dogs and humans in the City of Maringá to become infected with leptospires exists in both the central and the peripheral areas, a fact that reinforces the relevance of this study and of continuous epidemiological and environmental surveillance actions to control the disease in animals and in humans.

Keywords: Leptospirosis. Stray dogs. Risk areas.

RESUMO

Introdução: Leptospirose é uma zoonose que tem como hospedeiros primários os animais silvestres, sinantrópicos e domésticos. Os humanos comportam-se como hospedeiros terminais e acidentais. Sua prevalência depende dos animais portadores que disseminam o agente, de sua sobrevivência ambiental e do contato de pessoas susceptíveis. Cada sorovar tem um ou mais hospedeiros com diferentes níveis de adaptação. Os focos de leptospirose devem-se aos animais infectados, doentes e assintomáticos, considerados como fontes de infecção ambiental. O objetivo deste estudo foi determinar áreas de risco da infecção leptospírica em cães errantes e pacientes com diagnóstico de leptospirose nos anos de 2006 a 2008, em Maringá, Estado do Paraná, Brasil. Métodos: Foram estudados 335 cães errantes e 25 pacientes. Os soros, tanto dos animais como dos pacientes, foram examinados pela prova de soroaglutinação microscópica (SAM), para pesquisa de anticorpos antileptospíricos. Para determinar áreas de risco e a distribuição espacial da doença foram elaborados mapas temáticos. Resultados: Foram observados 41(12,2%) cães positivos para um ou mais sorovares de leptospiras, e os mais frequentes foram: Pyrogenes (43,9%), Canícola (21,9%) e Copennhageni (19,5%). Nos humanos, a positividade foi de 2 (8%) para os sorovares, Pyrogenes e Hardjo Prajitno e, Pyrogenes e Cynopteri. Conclusões: A análise espacial revelou que o risco de cães e humanos, no município de Maringá, se infectar com leptospiras está presente tanto em áreas centrais como periféricas, fato que reforça a relevância deste estudo e de ações contínuas de vigilância epidemiológica e ambiental para o controle da doença tanto nos animais como no homem.

Palavras-chaves: Leptospirose. Cães errantes. Áreas de risco.

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INTRODUCTION

Leptospirosis is a direct anthropozoonosis whose primary hosts are wild, synanthropic, and household animals. It is considered one of the major zoonotic diseases that can be caught in our environment. It is a generalized febrile disease caused by pathogenic spirochetes of the *Leptospira* genus, and its geographic distribution is strongly related to environmental issues and the process of city urbanization¹.

Pathogenic leptospires colonize the renal tissue of mammals and other animals as natural hosts, in which the disease is asymptomatic¹⁻³. They are also pathogenic to other mammals, including dogs and humans, which are accidental hosts⁴.

Household and wild animals may carry leptospires and contribute to their dissemination in nature. Transmission mainly occurs by contact with contaminated water and the urine of infected animals. Animals living in urban areas where sanitation and infrastructure conditions are unfavorable, with open dumping grounds, the absence of sewers, excessive amounts of discarded materials and food remains, and co-existence with other animal species, increase the risk factors.

The study of canine populations aims to acknowledge canine leptospirosis as a public health risk due to its potential to infect humans resulting from the great proximity established between men and dogs. In this regard, dogs can be considered as sentinel animals when studying the seroprevalence of infection in this species. Thus, the results may indicate risk factors for the human disease.

Leptospirosis has a great impact due to its relations with environmental factors, such as the disorderly growth of large urban centers and medium-sized cities, migration, poor basic sanitation conditions, and dump accumulation without adequate final disposal, which lead to an increase in the population of rodents. The survival capacity of leptospires in nature and the diversity of serovars and host species must also be taken into account.

The prevalence of this disease depends on a carrier animal, which is the disseminating agent,

on its survival in the environment, and on contact with susceptible individuals. Several animals can be hosts or reservoirs, and each serovar has one or more hosts with different adaptation levels. The permanence of leptospirosis focuses is on infected, sick, and asymptomatic animals, which are considered permanent sources of infection and environmental contamination⁵.

All cases must be mapped so that the spatial distribution of the disease and risk areas can be known, thus enabling the identification of risk areas and of increased incidence in humans and/or animals. Knowledge of the spatial and temporal variation of disease incidence and of aggravation determinants related to specific environmental situations provides support to the planning of preventive actions and health surveillance⁶.

This study aimed to investigate and present epidemiological findings of leptospirosis in humans and stray dogs in the City of Maringá, Paraná, Brazil, with the purpose of determining risk areas as well as ecological factors that may increase the incidence of leptospirosis.

METHODS

The City of Maringá is geographically located in the northwestern region in the State of Paraná. It has two climate types: tropical and subtropical. The average annual temperature during the study period was 22.2°C to 22.6°C, and the average rainfall was between 130.1 and 131.8. Its growth from early 1947 followed an urban development plan, and a planned city arose. The city has 17 parks with more than 240 hectares of native forests, which, added to the thousands of trees on its streets, avenues, and squares, provide 26.65m² of green area per inhabitant, thus earning its designation as a green city.

According to the demographic census⁷, the city had an estimated population of 335,512 inhabitants in 2009. The urban population corresponds to 97.4%, the rural population to 2.5%. To assess the canine population in the city, a census was organized by the Management of Environmental Surveillance and Zoonoses of the Health Department of Maringá and was taken by environmental health agents in 2006. The agents visited 150,386 properties, including residences and business facilities, in addition to strategic sites, such as scrap heaps, factories, and tire repair shops. In the aforementioned census, 51,670 household dogs were found. Taking into account that 5% of the dog population can be considered stray, such figure means that there were approximately 2,853 stray dogs in the city.

The studied population consisted of 355 stray dogs, which accounted for 12.3% of the estimated stray dogs in the urban area of Maringá from 2006 to 2008. Street dogs captured by the technical team of the Zoonosis Control Center of the city's Health Department were investigated. The human cases were analyzed prospectively in 25 male and female patients above five years old, suspected of having acute hemorrhagic icteric fever syndrome and who were notified and investigated by the Epidemiological Surveillance in 2008, the search for positive cases of leptospirosis from 2006 and 2007 having also been considered.

Serological examinations, conducted on both dogs and humans through the microscopic serum-agglutination technique, were used as antigen suspensions of leptospiral serovars produced and given by the Zoonosis Control Center of São Paulo and were performed by the Diagnosis Service of the Veterinary Medicine and Animal Husbandry

School of the Veterinary Hospital of Universidade Estadual Paulista (UNESP) at Botucatu, São Paulo, Brazil, according to guidelines by the Ministry of Health⁸. Suspensions of the following leptospire serovars were used as antigens: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Djasiman, Sentot, Gryppotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhaghiae, Javanica, Panamá, Pomona, Pyrogenes, Hardjo, Hardjo prajitno, Hardjo miniswajezak, Hardjo CTG, Hardjo bovis, Wolffi, Shermani, Tarassovi, Andamana, and Patoc.

For cartographic presentation, thematic maps/charts or quantitative cartograms were designed. They were punctual, and they spatially showed numeric data focusing on the distribution of a variable; in other words, they showed how much a certain variable was present in an area. The Geographic Information System and the ArcView 3.0 software (ERSI) were used for data mapping. The census zone proposed by IBGE for the census taken and adopted by the Maringá City administration was considered as a geographic unit⁷. To indicate the different themes on the map, the site of occurrence belonging to a certain census zone was taken into account.

Ethical considerations

This study was submitted to analysis and approved by the Ethics Committees for Research on Human Beings and Animals of UNESP, Botucatu, São Paulo..

RESULTS

The thematic chart (**Figure 1**) shows the distribution of 335 stray dogs captured in the urban area from 2006 to 2008, indicating the presence of dogs and its probable relation to the occurrence of human and canine leptospirosis. The spatial distribution of animals is punctual according to capture sites and is distributed by census zones in the respective urban sectors of central areas, such as the closest areas to the boundaries of the urban perimeter. The figure shows the presence of stray dogs in both central areas and those closest to the boundaries of the urban perimeter.

In **Figure 2**, the spatial distribution of infection occurrence in 41 (12.2%) stray dogs captured by the Maringá Zoonosis Control Center can be observed. Infected animals were found in central areas provided with better urban infrastructure, in peripheral areas, and in areas close to the boundaries of the city's urban perimeter.

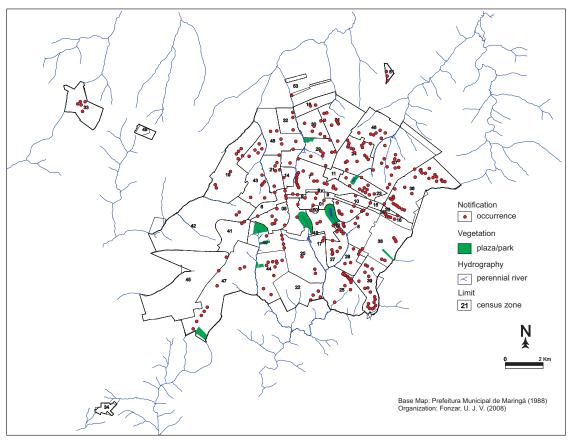
Table 1 shows the results of the serological response of the dogs examined in the different studied sites.

It was considered that the same animal could be infected with more than one leptospire serovar.

Of the 335 captured dogs, 41 (12.2%) were positive for one or more leptospire serovars, with titers equal to or higher than 100. The serovars found were Pyrogenes (18/41: 43.9%), Canicola (9/41: 21.9%), Copenhageni (8/41: 19.5%), Bratislava and Gryppotyphosa (2/41: 4.9%), and Hardjo and Pomona (1/41: 2.4%).

It can be observed that the animals responded to the different serovars studied; differences in serological response in relation to the titer for the different serovars presented can also be noted.

Figure 3 shows the spatial distribution of five human leptospirosis cases diagnosed in the study period. It is worth noting that they were distributed in central areas provided with better urban infrastructure, in peripheral areas, and in areas in the boundaries of the city's urban



 $FIGURE\ 1-Spatial\ distribution\ of\ the\ 335\ stray\ dogs\ captured\ in\ the\ urban\ perimeter\ of\ Maring\'a,\ State\ of\ Paran\'a,\ Brazil,\ from\ 2006\ to\ 2008.$

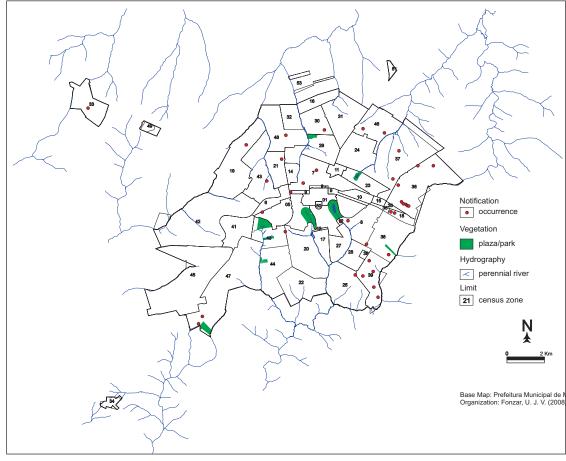


FIGURE 2 - Spatial distribution of leptospiral infection in 41 stray dogs in the urban perimeter of Maringá, State of Paraná, Brazil, from 2006 to 2008.

TABLE 1 - Serological response to leptospirosis in 41 dogs captured in Maringá, State of Paraná, Brazil, according to the microscopic seroagglutination technique from 2006 to 2008, Botucatu, State of São Paulo, Brazil, 2010.

		Antibody titer					
	100	200	400	800	1,600	Total	
Reagent serovars/titration	n	n	n	n	n	n	%
Bratislava	2	-	-	-	-	2	4.9
Canícola	2	1	3	2	1	9	22.0
Copenhageni	2	4	2	-	-	8	19.5
Gryppotyphosa	1	-	-	1	-	2	4.9
Hardjo	1	-	-	-	-	1	2.4
Pyrogenes	11	3	2	2	-	18	43.9
Pomona	-	-	-	-	1	1	2.4
Total	19	8	7	5	2	41	100.0

perimeter. Among the positive cases, 60% were female and 40% were male. When stratified by age group, four cases were concentrated between 20 and 46 years and one after 60 years. Their professions were collector of recyclables, health professional, hairdresser, and retired, all reported having had contact with water in the habit of fishing, through debris, and by having a dog at home. It is worth noting that it was possible to identify serovars of leptospira in only two positive cases in 2008, which were investigated within the purpose of this research as notification of acute febrile icterohemorrhagic syndrome. The patients were positive for the serovars Pyrogenes, Hardjo prajitno, and Prajitno Cynopteri.

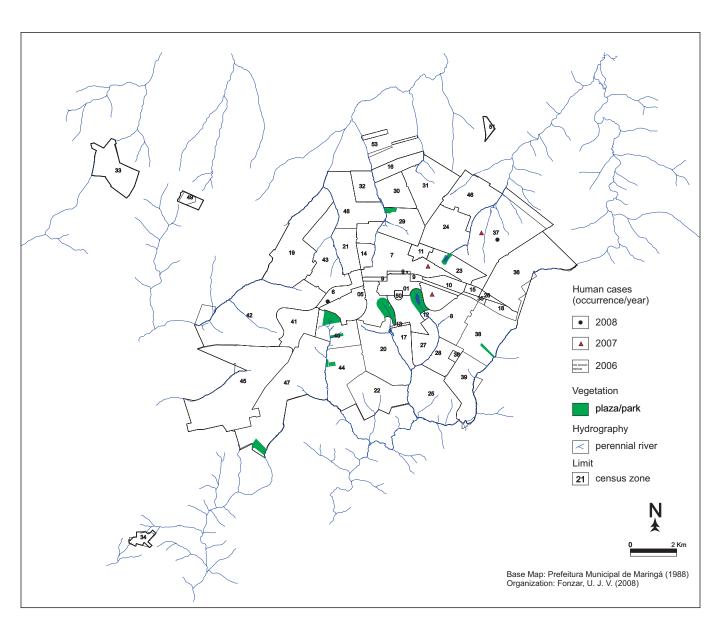


FIGURE 3 - Spatial distribution of five human leptospirosis cases in the urban perimeter of Maringá, State of Paraná, Brazil, from 2006 to 2008

DISCUSSION

In terms of the seroprevalence of canine leptospirosis, it was observed in this study that the serovars found were in agreement with those found in various studies in the literature on this subject. Of the 335 stray dogs captured, 41 (12.2%) showed anti-leptospire antibodies, with titers equal to or higher than 100. The serovars found, in order of prevalence, were Pyrogenes, Canicola, Copenhageni, Bratislava and Gryppotyphosa, and Hardjo and Pomona.

As to serological response for more than one serovar, the study showed that 9.7% (4 dogs) responded to more than one serovar, and the most frequent serovars were Pyrogenes and Canícola in 50% of such animals, Copenhageni and Pomona in 25%, and Gryppotyphosa and Pyrogenes in the other 25%.

These findings corroborate the reports by Favero et al. ⁹ Vasconcellos et al. ¹⁰ and Favero et al. ¹¹ who discussed that, in Brazil, the relationships between leptospire serovars and the most frequent preferential hosts vary according to region, and that the serovars Canícola, Icterohaemorrhagiae, and Copenhageni are the most prevalent.

It should be pointed out that in the present study the serovar Icterohaemorrhagiae was not found in the analyzed samples from dogs. This serovar is common in rats and is responsible for outbreaks and epidemics, particularly in tropical regions with high rainfall rates¹². The absence of this serovar may be related to the atmospheric conditions (bad weather) observed in the city, such as temperature, rain, wind and moisture. It is observed that in Maringá, even in periods of intense rainfall, no flooding occurs, which, therefore, reduces the possibility of infection with serovar Icterohaemorrhagiae¹².

When the geographic distribution of serologically positive dogs in the urban perimeter was evaluated, it was observed that the concentration of cases in the northern part of the city might be associated with various factors, such as the population density of stray dogs and people, and the existence of large empty urban areas and valley bottoms with grazing equines. In this northern part of the city, the urban network is denser, with a larger number of buildings and a concentration of housing complexes for low- and middle-income dwellings.

The presence of animal-drawn vehicles has also been observed. Such vehicles are very common in this area and are used by the population of waste collectors and informal workers who collect recyclable products and remove various dump types.

A larger industrial concentration and low population density have been observed in the southern part of the city. The concentration of cases in dogs in the city's southern area may be associated with the concentration of rodents from two environment types: the presence of valley bottoms and empty urban areas, and peripheral areas close to the rural zone.

Variability between serovars, such as that between Gryppotyphosa, Bratislava, Hardjo, and Pomona, can also be observed in this study. This may be associated with the dissemination of leptospires in the environment by equines and bovines and other possible wild animals that interact in the city's empty urban areas as well as in the boundaries with the City of Sarandi.

The cases of reagent dogs to serovar Grypptyphosa may be associated with the presence of skunks found in the city's valley bottoms, which show both secondary invasive vegetation, as in the case of Leucenas, and secondary or remaining native vegetation.

Equines and bovines are also considered as posing risk, since they share the same urban space with precarious sanitary and limited conditions of urban infrastructure, and the presence of organic garbage and an open air dump, among other materials. Additionally, the proximity with other animal species points out their interactions in the epidemiological chain of the disease¹³, although such less prevalent serovars found in the present study corroborate similar results observed in studies on bovines and equines^{9,14,15}.

Knowledge about predominant serovars in equines found in the environment is essential, since equines are maintained in the empty urban areas and in the boundaries of the urban perimeter to be used in animal-drawn vehicles for the freight of goods, construction materials, and recyclable waste, among others. They live with their owners in areas where there are rodents, stray dogs, community dogs, and other wild animals. These animals can be important sources of infection to other animals and humans.

Furthermore, wild animals, equines, and bovines contribute to the maintenance of their respective serovars in their natural habitat. The exchanges occurring in human and animal ecology during the colonization of a certain area can foster the transmission of this zoonosis to humans, since these exchanges allow for contact of the latter and household animals with the original focuses of infection. Deforestation and occupation by humans alongside the introduction of household animals in new areas tend to originate an ecosystem formed by different biocenoses that can influence the spread of zoonoses and the dissemination of other infectious diseases¹⁶.

With regard to the five cases of human leptospirosis confirmed in the city from 2006 to 2008 and investigated by the Epidemiological Surveillance, it was only possible to identify the serovars in two evaluated samples among the 25 patients investigated in 2008. It is worth nothing that, in 2006, there were no reports of the disease to the Epidemiological Surveillance Service in the city. The three positive cases in 2007 were considered from the samples sent to the Epidemiological Surveillance, which used the Central Laboratory of the State of Paraná, Department of Health as reference. This suggests the underestimation of laboratory diagnosis for the disease, which results in the lack of knowledge of prevalent serovars in the city, and consequently, the interactions in the link of the epidemiological chain of the disease and the possible relations between dogs, humans, and the rodents living together in the same environment.

Another noteworthy fact in the epidemiology of the disease in humans is that clinical cases are often not diagnosed and, therefore, not reported to the epidemiological surveillance services, thus showing unawareness and the need to establish, at the moment of patient anamnesis, the epidemiological connection of the disease and the association of risk factors during epidemiological investigation as well as the population groups that are most vulnerable to the infection.

This fact may be associated with the reason why many health care professionals do not value clinical procedures and do not include leptospirosis in differential diagnoses or why they do not adopt such procedures as a protocol determined by the epidemiological surveillance in case of clinical suspicion of hemorrhagic icteric fever syndrome, a fact that we consider to be regrettable given the importance of the disease in the public health scenario.

Of the leptospirosis-reagent human samples, the first patient was identified as positive for the disease for serovars Pyrogenes, with a titer of 3,200, and Hardjo Prajitno (200), whereas the second patient was reagent for serovars Pyrogenes (100) and Cynopteri (200).

In the first case, the diagnostic hypothesis was for hepatitis and dengue fever, later showing negative serology for the two diseases. The second patient was evaluated with clinical suspicion of acute hemorrhagic icteric fever syndrome, had negative serology for hantavirus, and progressed to death due to sepsis and bronchopneumonia. The same patient showed a throat neoplasia, in addition to being hypertensive and diabetic. According to the epidemiological investigation of the two cases, the presence of dogs in the household and a relation with fishing practices were observed. These two cases reflect the importance of valuing the epidemiological surveillance of leptospirosis in humans as a differential diagnosis among other pathologies.

The spatial distribution of the five human cases in the evaluation period showed the same pattern found for dogs, which were present in both central and peripheral areas as well as in those areas near or in the boundaries of the urban perimeter, thus reflecting on the city's characteristics. In this context, epidemiological and environmental surveillance applied to zoonoses must establish that, to better understand the health-disease process in any population, it is necessary to understand human beings and their interaction with the canine population in the physical, biological, social, and economic realms. These realms are considered determinant and conditioning factors in the health-disease process, since they establish the occurrence and prevalence of infectious and parasitical diseases in the environment¹⁷.

Information visualization is extremely useful to produce hypotheses, questions concerning associations between the studied events and the possibilities of ecological analyses, such as strengthening the correlations between environmental factors and explanatory variables¹⁸. This study made it possible to observe that the risk of infection with the disease in dogs and humans is present in the city's whole urban perimeter. Such perspective aims at understanding the geographic space with the disease distribution and the location of the inter-relations with biological and ecological components.

Knowledge of canine and human populations that are vulnerable to zoonoses such as leptospirosis must be a priority in the various levels of health care, from primary care to medium- and high-complexity levels in the system, both in public and in private services. It must be emphasized in the agendas of public policies in the interfaces of health and the environment. Within this logic, the Medical Geography currently points to possible paths to be trodden between health care, and environmental and social factors, as well as their risk determinants, including the survival conditions of reservoirs and servovars of leptospires.

Understanding the process of organization of geographic space by the population at different times and places is a peculiar form to understand diseases. Therefore, to Medical Geography, understanding such process is essential, since it allows for comprehending the role played by geographic space in the genesis and distribution of diseases so that health surveillance programs can be established according to a determined geographic space.

The risk for dogs and humans in the City of Maringá to become infected with leptospires is present regardless of degraded socio-environmental and ecological conditions. This reinforces the relevance of this study and the continuous epidemiological and environmental surveillance actions to control leptospirosis in both animals and humans. This study made it possible to identify the leptospiric canine and human infection, as well as to verify the presence of *Leptospira* in stray dogs present as accidental hosts in the urban environment and the vulnerability of human disease. The survey allowed the assessment that the risk of the disease, in dogs and humans, is present in the entire urban perimeter.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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