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Antibody prevalence of *Leptospira* spp. and *Brucella abortus* in domestic cats from Araguaína, Tocantins, North Region of Brazil*

Prevalência de anticorpos para *Leptospira* spp. e *Brucella Abortus* em gatos domésticos de Araguaína, Tocantins, Região Norte do Brasil

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Abstract

Leptospira spp. and *Brucella abortus* are bacterial pathogens that can infect humans and animals. The present study aimed to detect anti-*Leptospira* and anti-*B. abortus* antibodies and verified the presence of factors associated with seropositivity in cats. One hundred and eighty serum samples were collected from domestic cats (*Felis silvestris catus*) from the urban area of the municipality of Araguaína-Tocantins by phlebocentesis of the cephalic and jugular veins. The samples were subjected to detection of anti-*Leptospira* and anti-*B. abortus* antibodies, respectively, by microscopic seroagglutination and buffered acidified antigen testing, followed by confirmation by the 2-mercaptoethanol test and slow seroagglutination in tubes. Data from the epidemiological questionnaire (the age, sex, origin, breed, and presence of clinical signs) were analyzed using Epi Info® software with seropositivity data found to search for associated factors using the chi-square test. In the present study, the prevalence of *Leptospira* spp. was 5.56% (10/180). However, no sample was reactive to *B. abortus.* None of the studied variables were associated with seropositivity for the pathogens evaluated. Therefore, there is contact between *Leptospira* spp. and the feline population of the municipality, indicating the possibility of the circulation of pathogenic serovars and that the presence of anti-*Leptospira* antibodies does not depend on the variables analyzed.

Keywords: Brucellosis. Felines. Leptospirosis. Microscopic seroagglutination.

Resumo

Leptospira spp. e *Brucella abortus* são patógenos bacterianos que podem infectar humanos e animais. O presente estudo teve como objetivo detectar anticorpos anti-*Leptospira* e anti-*B.abortus* e verificar a presença de fatores associados com a soropositividade em gatos. Foram coletadas 180 amostras de soro de gatos domésticos (*Felis silvestris catus*) da zona urbana do município de Araguaína-Tocantins por flebocentese das veias cefálica e jugular. As amostras foram submetidas à detecção de anticorpos anti-*Leptospira* e anti-*B. abortus*, respectivamente, por soroaglutinação microscópica e teste do antígeno acidificado tamponado, seguido de confirmação pelo teste de 2-mercaptoetanol e soroaglutinação lenta em tubos. Os dados do questionário epidemiológico (idade, sexo, procedência, raça e presença de sinais clínicos) foram analisados no software Epi Info® com os dados de soropositividade encontrados para pesquisa de fatores associados pelo teste do qui-quadrado. No presente estudo, a prevalência de *Leptospira* spp. foi de 5,56% (10/180). No entanto, nenhuma amostra foi reativa para *B. abortus.* Nenhuma das variáveis estudadas foi associada com a soropositividade para os patógenos avaliados. Portanto, há contato entre *Leptospira* spp. e a população felina do município, indicando a possibilidade de circulação de sorovares patogênicos e que a presença de anticorpos anti-*Leptospira* independe das variáveis analisadas.

Palavras-chave: Brucelose. Felinos. Leptospirose. Soroaglutinação microscópica.

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Introduction

The species *Felis silvestris catus* is considered one of the most common companion animals in human populations, and its domestication is mainly related to rodent control (HU et al., 2014). The domestic feline population is approximately 22.1 million cats in Brazil (BRASIL, 2015). The contact between humans and cats brought with it a high probability of zoonose transmission (PUTZ and NALLY, 2020). Among the etiologic agents of zoonoses are *Leptospira* spp. and *Brucella* spp.

Leptospira is a bacterial genus in which 64 species have been described (VINCENT et al., 2019). The main form of transmission of *Leptospira* spp. in cats occurs via the oral route, especially by predation of rodents (SHOPHET and MARSHALL, 1980; DORSCH et al., 2020). The main clinical signs of feline leptospirosis are fever, interstitial nephritis, azotemia, kidney failure, polyuria, polydipsia, and hypergammaglobulinemia (ARBOUR et al., 2012; BEADU-LANGE and LANGE, 2014). Therefore, clinical identification of leptospirosis is very rare because your signals are nonspecific and clinical presentation can occur from subclinical or mild (MURILLO et al., 2020a). The role of cats in the epidemiology of leptospirosis is not known; however, it must have a heavy influence on the environmental characteristics of the studied region. No reports of the transmission of *Leptospira* infection from cats to humans have been described; however, it is possible for such an infection to occur due to feline leptospiruria (SPRISSLER et al., 2019; ALASHRAF et al., 2020).

Brucella abortus can also affect felines but is rarely studied. Its role in the epidemiology of human and animal brucellosis is still unknown (HARIHARAN e HARIHARAN, 2017). The detection of *Brucella* spp. DNA and the isolation of *B. abortus* in cat samples were recently reported (TRUONG et al., 2011; WARETH et al., 2017).

Antibody research against *B. abortus* and *Leptospira* spp. in felines in Brazil is rare, particularly in the state of Tocantins. This demonstrates the importance of studies focused on the circulation of such pathogens to guide preventive and control measures.

The present study aimed to investigate the presence of antibodies against *Leptospira* spp. and *B. abortus* and analyze potential risk factors associated with seroprevalence in domestic cats in the state of Tocantins.

Material and Methods

The municipality of Araguaína (7° 11’ 28″ S and 48° 12’ 26″ W) is located near the border with the states of Pará and Maranhão at an altitude of 227 m. It has a humid climate classified as Aw (tropical with summer rains), average annual precipitation ranging between 1700–1800 mm, an average annual temperature of 27–28 °C and a maximum of 32 ºC (PENEREIRO et al., 2016). Araguaína had an accelerated urbanization process due to demographic growth after the 1970s, generating deficiencies in the city’s infrastructure. Therefore, Araguaína still concentrates many preserved wooded areas contiguous to the urban space, which often generates the approximation of wild animals with animal households (SANTOS et al., 2013).

The present study was approved by the Animal Use Ethics Committee of the Federal University of Tocantins (Protocol No. 23101.000.988/2018-10).

Sampling was performed based on the expected prevalence of 6.96% for *Leptospira* spp. and 2.1% for *B. abortus*, at 95% confidence level, variation of 5%, assuming an estimated population of 16,607 domestic cats (PARREIRA et al., 2010; ARAGUAÍNA, 2017; GAROUSSI et al., 2018). Based on the methodology recommended by Thrusfield and Christley (2018), the sample size was estimated to be at least 99 animals. However, as the Animal Use Ethics Committee authorized the collection of a large sample size for another study with different pathogens, we opted to analyze 180 samples.

Blood samples from 180 cats (107 domiciled and 73 strays) were collected from 2015 to 2018 by phlebocentesis (from the cephalic or jugular veins) in tubes with coagulation activator and tubes with anticoagulant in a vacuum suction system. Sterilized polystyrene microtubes were used to store the serum from animals at -20 °C.

Once the animal was captured and physically contained, blood samples were collected, clinical examination was performed, and vital signs were checked. All animals were sampled from the urban area of the municipality.

Samples of the stray animals were obtained by random capture by the Center for Zoonosis Control of Araguaína and in the vicinity of the School of Veterinary Medicine of the Federal University of Tocantins.

The owners of the domiciled cats were informed about the main aspects of the research and the collection of biological samples of their animals was authorized by signing the consent form. The epidemiological variables collected were the race, sex (male or female), age group (≥6 months or <6 months), breed (with or without breed), origin (domiciled or stray), and presence or absence of clinical signs at clinical examination. Epidemiological data from stray animals were selected only through clinical examination.

Microscopic seroagglutination (MAT) was used to detect antibodies against *Leptospira* spp. This is considered a standard test, using live cultures of *Leptospira* spp. grown in Ellinghausen, McCullough, Johnson, and Harris medium in a biological oxygen demand incubator at 30 °C for 7 to 10 days. The following 20 serovars were used: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Canicola, Djasiman, Sentot, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Copenhageni, Cantagalo, Pomona, Pyrogenes, Hardjoprajitno, Hardjobovis, Wolffi, Shermani, and Tarassovi. The lack of previous data on circulating serovars in other animals in the urban and rural areas of Araguaína made it impossible to include other serovars and serogroups.

The samples were subjected to a screening step that consisted of inserting 50 μL of serum (sample to be tested) and 50 μL of antigen diluted in the saline buffer into each well of the serological test microplates for 1 h at 37 °C and subsequently read in a dark field microscope (Zeiss ® - model Axioskop 40) using the 10× objective lens. The sera were tested at an initial dilution of 1:100 as a cut-off for positivity (100 IU) and a final dilution of 1:800. Samples that agglutinated 50% or more free leptospires were considered reagents in comparison to the negative control in a dark-field microscope (Hässle et al., 2019).

Furthermore, antibodies against *B. abortus* were investigated by performing the Rose Bengal test with buffered acidified antigen (BAA) provided by Instituto Biológico®, using *B. abortus* strain 1119-3 antigen buffered at pH 3.65, at 8% concentration.

In a glass plate, 30 µL of serum and 30 µL of antigen were homogenized. After 4 min of constant homogenization, the presence or absence of agglutination in the lumps was verified using indirect light. Samples that demonstrated agglutination for up to 4 min were considered to be positive and were standardized. The samples considered reactive in the BAA test were subjected to a confirmatory test of 2-Mercaptoethanol (2-ME) in conjunction with the slow seroagglutination test in tubes.

The analysis of risk factors was based on the association of the presence or absence of *Leptospira* spp. and *B. abortus* infection in the cat population with the studied epidemiological characteristics. The two-tailed chi-square ($\chi 2$) statistical method with Yates correction and odds ratio was performed using *EpiInfo*™ 7.2 statistical software. A value of $p \leq 0.05$ was considered as a statistically significant variable.

Results and Discussion

Antibodies against *Leptospira* spp. were found in 5.56% (10/180) of the animals. (Table 1).

Table 1: Prevalence of antibodies against *Leptospira* spp. in 180 samples of cat serum from Araguaína municipality, state of Tocantins, 2019, according to the titer tested.

Serovar	Antibody Titer (IU)				Positives (%)	CI 95%
	1:100	1:200	1:400	1:800		
Pomona	2	2	2	2	8 (4.44%)	
Djasiman	1	0	0	0	1 (0.56%)	
Grippotyphosa	1	0	0	0	1 (0.56%)	
Total	**4**	**2**	**2**	**2**	**10/180 (5.56)**	**2.7-10.0**

CI: confidence interval

The prevalence observed in this study was low and was similar to those previously described in Pantanal, Mato Grosso do Sul (10%, 1/10), Goiânia, Goiás (6.96%; 23/330), Patos, Paraíba (5.43 %; 7/129), and Curitiba, Paraná (4.6%; 3/65) using the same MAT technique (PARREIRA et al., 2010; BRASIL et al., 2014; FURTADO et al., 2015; CORDEIRO et al., 2017).

The most prevalent serovar found in this study was Pomona, which is similar to that found in studies carried out in Montreal, Canada, Patos, and Paraíba (BRASIL et al., 2014; RODRIGUEZ et al., 2014). In Montreal, serovar Grippotyphosa was also one of the most prevalent in serological responses (RODRIGUEZ et al., 2014). Seroreactive cats to different serovars have been observed in other locations in Brazil, which may be related mainly to different environmental characteristics. In contrast to the results of the present study, in Goiânia and Goiás, there was a greater number of seroreactive samples in serovar Cynopteri, while in Pantanal and Mato Grosso do Sul, they were detected only in serovar Hardjo (PARREIRA et al., 2010; FURTADo et al., 2015). However, it is noteworthy that in Goiânia and Goiás, serum-reactive samples were also detected in serovar Djasiman, similar to the findings of the present study (PARREIRA et al., 2010).

The six domiciled animals seroreactive for *Leptospira* spp. were from socially vulnerable neighborhoods, peripheral and close to rural areas. All animals in the urban area were positive only for serovar Pomona. Only two seroreactive stray animals in the study were seropositive for the Pomona serovar, while two other stray animals had a positive reaction for serovars present in wild animals, suggesting the participation of other epidemiological factors. Pigs are considered reservoirs of the Pomona serovar; however, although prevalent, it is not common to raise pigs in the urban area of Araguaína, Tocantins. This suggests the participation of other hosts, such as dogs that can be naturally infected by this serovar (FAINE et al.,1999). In addition, the participation of rodents and bats is not ruled out, as they are common prey for felines in urban areas. In Brazil, seroreactivity has already been verified for the Pomona serovar in synanthropic rodents and other serovars in bats (ZETUN et al., 2009; LENHARO et al.2012). Unlike the Pomona serovar, cats are believed to be more resistant to infections by serovars commonly present in synanthropic rodents, such as Icterohaemorrhagiae and Copenhageni. This resistance may be due to the coevolution of cats with rodents that had established an adaptation for these serovar infections (LILENBAUM et al., 2014).

It has been suggested that cats are susceptible to infections by the Pomona serovar, as this serovar has already been isolated from the urine culture of a feline patient in Chile and related to clinical cases of feline leptospirosis in the United States of America (ARBOUR et al., 2012; OJEDA et al., 2018). Therefore, seroreactivity for the Pomona serovar indicates a possible occurrence of leptospirosis in cats, suggesting the inclusion of this disease in the clinical suspicion and the differential diagnosis in feline medicine.

Serovars Grippotyphosa and Djasiman are closely related to wild animals (FAINE et al., 1999). It is important to note that in the municipality of Araguaína, there are many human dwellings with domestic animals that are close to preserved areas because of the rapid urban expansion, especially after the opening of the BR-153 highway. Therefore, the urban and rural areas of the municipality of Araguaína have environmental similarities.

It is still necessary to be careful when establishing the role of the cat in the transmission of leptospirosis, as it appears to have a certain resistance to infection and other animals have an established role in epidemiology, such as rodents that develop leptospiruria for a long time and with a high bacterial load (COSTA et al., 2015; SILVA et al., 2020).

The epidemiological variables analyzed were not confirmed as factors associated with the detection of antibodies against *Leptospira* spp. (Table 2).

Table 2: Univariate analysis of the risk of infection by *Leptospira* spp. in domestic cats (N=180) from the municipality of Araguaína, Tocantins, Brazil, 2019 and epidemiological variables.

Variable	Total	*Leptospira* spp. Positives (%)	OR	CI 95%	p-value	χ^2
Age						
<6 months	30	2 (6.7%)	0.789	0.18–5.82	0.844	0.021
≥6 months	150	8 (5.3%)				
Sex						
Male	81	4 (4.9%)	1.242	0.22–2.96	>0.99	0
Female	99	6 (6.1%)				
Clinical signs						
Present	51	3 (5.9%)	1.089	0.22–4.32	0.809	0.058
Absent	129	7 (5.4%)				
Origin						
Stray	73	4 (5.5%)	0.976	0.26–3.58	0.768	0.087
Domiciled	107	6 (5.6%)				
Defined Breed						
Yes	8	1 (12.5%)	2.567	0.10–19.37	0.9301	0.007
No	172	9 (5.2%)				

OR: Odds ratio. CI: Confidence interval.

Age and sex were not studied in previous studies (SPRISSLER et al., 2019; MURILLO et al., 2020b; SILVA et al., 2020). The absence of associated variables differed from that found by Brasil et al. (2014), Rodriguez et al. (2014), Sprissler et al. (2019), Dorsch et al. (2020), and Lehtla et al. (2020), who demonstrated a statistically significant association between the variables age (older cats) and free lifestyle (without mobility restrictions) and infection by *Leptospira* spp. The clinical signs present in the three seroreactive animals were fever, lethargy, anorexia, and opaque hair. Renal clinical signs could be likely to be associated with *Leptospira* spp.infection, as well as the inclusion of an increased number of animals with clinical signs in the study (RODRIGUEZ et al., 2014).

The low prevalence and impossibility of adequate distribution of the population in the sample reduced the statistical power to detect significant variables. The inclusion of a group of stray animals in the sample allowed for a comparative epidemiological study with the urban group but made it impossible to include other variables, such as the type of food, hunting habits, vaccine application, access to outdoor areas, contact with other animals, and veterinary assistance.

Regarding the detection of anti-*B. abortus* antibodies, four animals (2.2%) were serum-reactive to the BAA screening test; however, seroreactivity was not demonstrated by the 2-ME test. It should be noted that cross-reactions can occur in screening tests with other microorganisms, such as *Yersinia enterocolitica* and *Escherichia coli* (Bonfini et al., 2018). Unlike what was found in this study, Garoussi et al. (2018) found 2.1% of cats with positive reactions for the detection of anti-*B. abortus* antibodies by BAA and 1.4% by 2-ME in Iran. The difference between the two studies may have been due to the specific characteristics of the present study, which only included animals from urban areas, while Garoussi et al. (2018) included cats from dairy farms, where contact with cattle that are considered the preferred host of *B. abortus* is likely.

The prevalence of *B. abortus* infection was expected to be low based on studies by other authors, since cats are considered resistant to infections by bacteria of the genus *Brucella* spp. and considered as accidental hosts (ACHA and SZYFRE, 2001). However, *B. abortus* has been isolated from uterine discharge from a cat in contact with a bovine resident from a dairy farm (WARETH et al., 2017).

Based on the results, it was found that the cats had been exposed to *Leptospira* spp., suggesting that cases of infection and clinical illness may occur in the animals of Araguaína. Considering the close interaction between dogs, cats, and humans, further studies must be carried out in the feline population, to provide information about this disease in animals (MURILLO et al., 2020b). A seroreactivity sample in microscopic seroagglutination does not necessarily imply that cats are considered reservoirs or carriers but only that they have been exposed to *Leptospira* spp. or are in a stage of latent infection (FORNAZARI, 2017).

Conclusions

The presence of antibodies against *Leptospira* spp. in the Pomona, Djasiman, and Grippotyphosa serovars was detected in the domestic feline population of the urban area of the municipality of Araguaína. The analyzed epidemiological variables (age, sex, origin, breed, and presence of clinical signs) were not associated with seropositivity for *Leptospira* spp. No sample had anti-*B. abortus* agglutinins. It is suggested that prophylaxis programs should emphasize, among other measures, rodent control and restriction of access from external areas, as a way of reducing exposure to *Leptospira* spp. Due to the epidemiology of leptospirosis and brucellosis involving human, animal, and environmental health, it is indicated to evaluate the interdisciplinarity of One Health approaches for efficient control of the pathogens.

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