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POSITIVITY FOR BRUCELLA CANIS IN SERUM FROM DOGS LOCATED IN TERESINA (PI, BRAZIL)

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ABSTRACT

Brucellosis is an infectious, contagious, cosmopolitan disease that affects domestic and wild animals and humans, which is why it is considered a zoonose. Brucellosis has not yet been diagnosed in dogs in Piauí (Brazil). For this reason, a study was carried out with serum from animals of reproductive age in the municipality of Teresina, capital of the State of Piauí (Brazil) with serum from dogs from the routine care of the Veterinary Hospital of the Federal University of Piauí, as well as from private clinics in the said city. Blood was collected from all animals for serological tests to detect anti-Brucella canis antibodies. 591 serum samples from non-castrated animals between one and seven years of age were used, without distinction of sex or breed. The samples were submitted to the serological test of immunodiffusion in agar gel using antigen extracted from the cell wall of Brucella ovis. Among the selected dogs, 44 animals were positive for Brucella canis, which represents a frequency of 7.44%. Most dogs were asymptomatic. Among symptomatic patients, lymphadenopathy was the most frequent finding. It was concluded that Brucellosis is present in the canine population of Teresina, but it has not yet been correctly diagnosed by veterinarians, which puts public health at risk.

Keywords: Brucellosis. Dogs. Zoonose.

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INTRODUCTION

In recent decades, there have been social transformations and urbanization of the human population, which has contributed to the increase in the number of dogs in developing countries[1]. (COLELLA, Vito et al. 2022) This increase results in greater interaction between dogs and people, especially children, the elderly and pregnant women, generating great concern regarding the health of these animals, as they can be carriers of various infectious diseases (zoonose) that can be transmitted to humans· (ISODA, 2024). Among the zoonoses transmitted by dogs, brucellosis is included, a neglected and little reported disease, mainly due to the non-specific clinical signs, which can easily lead the illness to be confused with other diseases· (BRASIL, 2023)

According to Greene (2015), the main cause of canine brucellosis is the bacteria *B. canis*. However, cases of dogs infected by three other species have already been recorded, such as *B. melitensis*, *B. abortus* and *B. suis*, in situations where dogs were in close contact and shared the same environment as cattle, goats, sheep and pigs. (AYOOLA, 2016). In the view of De Massis (2022), it is important to emphasize that, among all forms of brucellosis that affect domestic animals, canine brucellosis is the least common and also the least studied.

B. canis is a coccobacillary bacterium (with a size between 1.0 and 1.5 µm), facultatively intracellular, aerobic, gram-negative and with a rough surface. Susceptibility to infection by this bacteria is present only in domestic and wild dogs. Felines, on the other hand, are relatively resistant, with reports of infection only in experimental studies. (KEID, 2015).

Canine brucellosis is an emerging zoonotic disease. This infection is the main cause of reproductive problems in dogs and is endemic in many countries. (Hensel, 2018). As noted by Ashmi (2022), dogs infected by *B. canis* can present several reproductive disorders, with or without apparent clinical signs.

Dogs infected with *B. canis* may exhibit a variety of reproductive disorders, with or without overt clinical signs. In addition, this infection is also economically important, as its high frequency and occurrence in kennels cause damage to purebred dog breeders, due to reproductive losses. (Keid, 2023).

Serological tests are considered the best method of detecting *Brucella* infection. Among the serological tests widely used in the diagnosis of canine brucellosis caused by *Brucella canis*, the most common technique in Brazil and in other countries where the

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disease is present is AGID (immunodiffusion in agar gel). (HAFEMANN, 2018). This technique uses an antigen extracted from the lipopolysaccharide of *Brucella ovis*. Due to the sharing of antigens between *Brucella canis* and *Brucella ovis*, the same reagents can be used for the diagnosis of brucellosis in sheep and dogs. AGID has been widely applied and allows the detection of antibodies from eight to 12 weeks after infection, and can persist for several years. (Greene, 2015).

So far, no cases of canine brucellosis have been reported in Piauí, and the disease has not yet been diagnosed in the region. Therefore, it is necessary to carry out more comprehensive studies on this disease. The aim of this study was to investigate whether brucellosis occurs in dogs from Piauí, both in animals with clinical signs of the disease and in those without apparent signs. The search for antibodies against *Brucella canis* in serological tests was carried out, thus contributing to a better understanding of this zoonosis in the Metropolitan Region of Teresina.

MATERIAL AND METHODS

ANIMALS AND EXPERIMENTAL DESIGN

An epidemiological study was carried out in the city of Teresina, aiming to detect the presence of canine brucellosis in the metropolitan region of Teresina (PI), in dogs from the medical clinic of the Veterinary Hospital of the Federal University of Piauí, as well as from private clinics in the same city. Dogs were randomly selected, regardless of complaints or clinical signs. A total of 591 non-castrated animals, aged between one and seven years, were used, without distinction of sex or breed, with the authorization of the guardian and the approval of the Bioethics Committee (Protocol 99/12; Federal University of Piauí), in compliance with current regulations. Information on age, gender and breed was recorded individually, and the clinical examination was performed by veterinarians. For serological analysis, blood samples were collected by venipuncture of the jugular vein after antisepsis with 2% iodinated alcohol, using a vacuum system in tubes without anticoagulant, which were identified with information about the owner and the animal. The tubes were kept at room temperature for one to two hours for coagulation, then stored at 4°C overnight and then subjected to centrifugation at 2,500g for 10 minutes to obtain the sera, which were stored at -20°C.

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AGAR GEL IMMUNODIFFUSION TECHNIQUE

The agar gel immunodiffusion technique (AGID) was performed using an antigen obtained from the cell wall of *Brucella ovis* (Tecpar, Paraná, Brazil), as described in the literature (ALTON, 1988). At the Laboratory of Pathophysiology of Reproduction from the Federal University of Piauí. The agar gel was prepared with 1.2g of Noble agar, 5ml of Borate buffer pH 8.3 and 93ml of 10% NaCl. Then, 4.5% of the gel was distributed in Petri dishes. After solidification, the gel was perforated with rosettes containing six peripheral holes and one central one, with holes of 6 mm in diameter and distance of 2.5 mm between the edges. The holes were filled with the antigen, positive control sera and sera to be tested, and the plates were kept in a humid chamber at room temperature.

Readings were taken after 24, 48 and 72 hours, using an indirect lighting system and dark background. The interpretation was made by observing the formation of a precipitation line between the tested sera and the positive control sera, in contact with the antigen. Tested sera that showed precipitation lines identical to those formed by positive control sera were considered positive. The sera considered negative were those in which the formation of precipitation lines did not occur or the formed lines did not show identity with the positive control sera.

SAMPLE SIZE AND STATISTICAL ANALYSIS

The sample size was calculated using the Epi Info 7.0 program, considering an expected prevalence of 50% (representing a disease of unknown occurrence in a given population) in a population estimate of 89,729 dogs, with a confidence level of 95% and a margin of error of 5%. This resulted in a sample of 384 animals. However, in this particular study, 591 samples from dogs residing in the urban area of Teresina were used. The association between canine brucellosis and variables such as sex, age and breed of animals was analyzed using the Chi-square ($\chi 2$) test and Fisher's exact test, with a significance level of 0.05. All statistical analyzes were performed using GraphPad Prism software, version 6.0.

RESULTS AND DISCUSSION

The selection of samples carried out in this research is adequate to determine the presence or absence of the disease in the city of Teresina. The sample calculation considered an expected prevalence of 50% and resulted in a sample size of 384. However,

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in this particular study, an even larger number of samples was used, totaling 591. This strengthens the representativeness of the sample and allows obtaining more robust conclusions. It is important to mention that in previous studies the same sample calculation was used[16,17], reinforcing the consistency of the results obtained.

The test used in this research, agar gel immunodiffusion (AGID), was efficient in identifying positive dogs for the infection. This serological test is widely used in Brazil as an initial method to detect positive dogs for *Brucella canis* (HAFEMANN et al., 2018). Although the test used is considered reliable, there are reports of false positive and false negative results. (COSFORD, 2018). In the view of De Massis (2022), when there are no reproductive failures, the disease is difficult to diagnose. Clinically, *Brucella canis* is typically associated with reproductive abnormalities, but a variety of non-reproductive signs may occur. Therefore, the diagnosis of canine brucellosis is considered a challenge, even with the use of multiple tests. (Mol, et al., 2020; Santos, et al., 2021). In this sense, further research is suggested in Teresina using more sensitive and specific tests, such as PCR, complement fixation and bacteriology. (Cosford, 2018; Santos, et al., 2021; Almeida, et al., 2004).

Among the 591 canine serum samples tested using the AGID method, 44 were positive, indicating an infection rate of 7.44% in the city of Teresina (PI). This frequency was higher than other studies conducted in Brazil (Ferreira et al., 2007; Vasconcelos et al., 2008) and abroad (Laverde, et al., 2021), but it is close to the results of other Brazilian studies. (Cavalcanti et al., 2006).

Probably, the discrepancy in frequency found in this study compared to other studies is due to differences in the studied populations, geographic region and sampling methods used. To corroborate this hypothesis, it is observed that in a study with the same test method performed in a similar population sample, the result was very close to that found in this study (7.4%). (MORAES, 2002)

Among the 44 samples positive for *Brucella canis* infection, 20 (6.73%) belonged to male dogs and 24 (8.16%) to female dogs (Table 1). It was observed that there was no statistically significant difference in the frequency of animals reactive to the AGID test for canine brucellosis in relation to sex ($_{\chi}2=0.44$; gl=1; P=0.5) (Table 1). The lack of this statistical difference related to sex is common this disease among dogs. (CAVALCANTI, 2006). However, in this study, the absolute number of females was slightly higher. In two

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studies carried out in Paraná (Brazil), a higher prevalence of antibodies against *Brucella canis* was found in females. (Hafemann et al., 2018).

According to Ayoola (2016), this can be explained by the fact that males usually mate with several females during the reproduction period. In a Brazilian survey, the authors reported a higher frequency in males. However, the sample of male dogs was considerably larger than the number of females, which raises doubts about the reported results. (ALVES, et al., 2003).

In this study, dogs were categorized into age groups from 1 to 5 years and from 5 to 10 years for better organization and data analysis. The association between animal age and *Brucella canis* infection was evaluated in these age groups. Of the dogs between 1 and 5 years old, 8.86% (29/327) were seropositive, while in the age group 5 to 10 years old, 5.68% (15/264) of the dogs in the sample were seropositive (Table 2). No statistically significant difference was found between the age group of the dogs and the frequency of seropositivity to the AGID test for *Brucella canis* ($\chi 2$=2.15; gl=1; P=0.14) (Table 2). This result indicates that dogs of all ages are susceptible to infection by the bacteria. However, a small difference was observed between age groups, with a higher percentage of positives in the 1 to 5 years age group. Similar results were found in other studies. (Lingam, et al., 2020). And this is probably related to the fact that in this age group the animals are in their sexual maturity and they usually have contact with a greater number of sexual partners, facilitating the transmission of the infection[34]. (LALI, et al., 2021).

Table 1.Table 1. Frequency of dogs reactive to Agar gel immunodiffusion for canine brucellosis, according to sex, in Teresina, PI, Brazil (n=591)

Sex	% Frequency (+/n)	
	Positives	Negatives
Male	6.73 (20/297)[a]	93.26 (277/297)[a]
Female	8.16 (24/294)[a]	91.83 (270/294)[a]

+, number of positive animals; n, number of samples per variable a there was no significant difference (p>0.05) between sex, according to the $\chi 2$ test.

Table 2.Table 2. Frequency of dogs reactive to Agar gel immunodiffusion for canine brucellosis, according to age, in Teresina, PI, Brazil (n=591).

Age (years)	% Frequency (+/n)	
	Positive	Negative
1 – 5	8.86 (29/327)[a]	91.13 (298/327) [a]
5 – 10	5.68 (15/264) [a]	94.31 (249/264) [a]

+, number of positive animals; n, number of samples per variable

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[a] There was no significant difference (p>0.05) between age groups, according to the χ2 test.

No statistically significant differences were found in the association between breeds and animals reactive to the agar gel immunodiffusion test for canine brucellosis ($_{\chi}2=14.27$; gl=17; P>0.5) in the studied population sample (Table 3). Therefore, this study observed that there is no racial predisposition for the occurrence of canine brucellosis[25].

Table 3. Frequency of dogs reactive to Agar gel immunodiffusion (AGID) for canine brucellosis, according to breed, in Teresina, PI, Brazil (n=591).

Breed	AGID	Total (%)
	Reactive Animals (%)	
Mixed breed	20 (9.6) [a]	208 (35.2)
Poodle	7 (6.7) [a]	104 (17.6)
German Shepherd	2 (5.4) [a]	37 (6.3)
Pitbull	1 (3.1) [a]	32 (5.4)
Pinscher	1 (3.7) [a]	27 (4.6)
Rottweiler	2 (9.1) [a]	22 (3.7)
Cocker Spaniel	0 (0.0) [a]	18 (3.0)
Yorkshire	3 (18.8) [a]	16 (2.7)
Dachshund	2 (13,3) [a]	15 (2.5)
Labrador	0 (0.0) [a]	13 (2.2)
Pekingese	0 (0.0) [a]	12 (2.0)
Maltese	0 (0.0) [a]	11 (1.9)
Brazilian Fila	0 (0.0) [a]	9 (1.5)
Bulldog	1 (14.3) [a]	7 (1.2)
Dalmatian	0 (0.0) [a]	7 (1.2)
Shih Tzu	1 (14.3) [a]	7 (1.2)
Weimaraner	1 (16.7) [a]	6 (1.0)
Other breeds	3 (7.5) [a]	40 (6.8)
Total	44 (7.4) [a]	591 (100.0)

As for the clinical signs, of the 44 positive animals, it was observed that many were asymptomatic (38.63%; 17/44) (Table 4). Among symptomatic dogs (20.45%; 9/44), the majority (88.88%; 8/9) had only lymphadenopathy as a clinical sign. One bitch presented pyometra. In ten dogs (22.72%; 10/44), it was not possible to retrieve the clinical signs due to lack of complete information in the clinical records.

Table 4. Clinical signs observed in the seropositive canine population for canine brucellosis in Teresina, PI, Brazil (n=44).

CLINICAL SIGNS	FEMALE	MALE	TOTAL
Abortion	0	-	0
Asymptomatic	11	6	17
Lymphadenopathy	4	4	8
Orchitis	-	0	0
Pyometra	1	-	1
Scrotal dermatites	-	0	0

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No clinical data	5	5	10

As noted by Moraes (2002), he absence of clinical signs in most animals (Table 4) was observed in another study. This is probably due to the fact that the clinical signs of the disease in dogs are not very evidente, and when they do occur, they are usually nonspecific, although they are associated with reproduction, such as abortions. However, these clinical signs are not pathognomonic (KEID, et al., 2017).

Lymphadenopathy, observed as the only clinical sign in eight animals (Table 1), is common in the diseases. (MOL, et al., 2020; SANTOS, et al., 2021; KEID, et al., 2017) but it can also be present in other endemic diseases in Teresina (PI), such as visceral leishmaniasis and ehrlichiosis which makes the diagnosis of canine brucellosis difficult. Only one animal showed a clinical sign related to reproduction (pyometra). In studies carried out in Brazil, there is no mention of pyometra as a clinical sign of the disease (VASCONCELOS, et al., 2008). However, in a kennel study, a positive bitch had abortion and pyometra[40]. Probably due to the low incidence of brucellosis diagnosis in bitches with pyometra, most veterinarians do not associate the disease with pyometra in bitches, not considering Brucella as an etiological agent of pyometra.

CONCLUSION

Canine brucellosis is present in the canine population of Teresina and had not yet been reported or diagnosed until the present moment. It is important to highlight that most of the dogs tested in this study were asymptomatic for *Brucella canis* infection, making it even more difficult to diagnose the disease in the city. The frequency of 7.44% must be taken into account, as it indicates, even if small, that a portion of the canine population of Teresina may be acting as a reservoir for *Brucella canis*, exposing not only other dogs, but also humans to the risk of infection.

The serological test used in this research (AGID) may present false positive and false negative results. Therefore, it is important to consider this possibility of interference in the results, which leads to the conclusion that more in-depth research is needed for the development and standardization of more specific, sensitive and, preferably, fast, practical and economically viable technical procedures.

This study should serve as a warning and make veterinarians in Teresina aware of the importance of taking precautions when handling patients during the clinical examination,

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as well as considering canine brucellosis as part of the differential diagnosis of certain diseases, since animals may be asymptomatic. In addition, it is suggested that the serological test be incorporated into the routine of veterinary clinical services, not only in the municipality, but also throughout the state and even throughout the country.

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DISCLOSURE

The authors inform that there are no conflicts of interest.

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