Zoonotic smooth and rough *Brucella* in dogs: seroprevalence and associated factors in an Atlantic Rainforest area of the state of Paraíba, Northeastern Brazil

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ABSTRACT: *Canine brucellosis an infectious disease caused by bacteria of the genus Brucella, with world wide distribution and zoonotic impact, and in humans and animals is a neglected disease. In the present study, the sero prevalence of B. canis and B. abortus were determined in a probabilistic sample of housed dogs from the Atlantic Rainforest area of the state of Paraíba, Brazil, and the factors associated with sero positivity. A total of 386 dogs over three months of age were used. For the search for anti-B.canis antibodies the agar gel immune diffusion test (IDGA) was used as a screening and IDGA+2ME as confirmatory test, and to search for anti-B. abortus antibodies the Rose Bengal test (RBT) test was used. Apparent and real prevalences were calculated, and robust Poisson regression was used to identify factors associated with prevalence. The real prevalence fB. Canis was 12.6% and of B. abortus was 22.8%. The factors associated with sero positivity for B. canis were age greater than 10 years (prevalence ratio; PR = 6.38; P = 0.024) and dogs reared in they ard (PR = 5.20; P = 0.035) and for B. abortus was no treplacement of water of animals everyday (PR = 1.48; P = 0.033). It can be concluded that the prevalence of B. canis and B. Abortus in the region is high, which warns to the adopting of control and prevention measures, as well as greater care in the management of animals, especially for elderly dogs.*
Key words: *Brucella canis, Brucella abortus, public health, epidemiology, dogs.*

Brucella zoonóticalisa e rugosa em cães: soroprevalência e fatores associados em área de Mata Atlântica do estado da Paraíba, Nordeste brasileiro

RESUMO: *A brucelose canina é uma doença infecciosa causada por bactérias do gênero Brucella, com distribuição mundial e de caráter zoonótico, e em humanos e animais é uma doença negligenciada. No presente estudo foram determinados as soroprevalências de B. canis e B. abortusem uma amostra probabilística de cães domiciliados da área urbana de oito municípios localizados na região da Mata Atlântica do Estado da Paraíba, Brasil, e os fatores associados com a soropositividade. Foram utilizados 386 cães com mais de três meses de idade. Para a pesquisa de anticorpos anti-B. canis foi utilizado o teste de imunodifusão em gel de ágar (IDGA) como triagem e IDGA+2ME como confirmatório, e para a pesquisa de anticorpos anti-B. abortusfoi utilizado o teste do antígeno acidificado tamponado (AAT). Foram calculadas as prevalências aparente e real, e para a identificação de fatores associados com a prevalência foi empregada regressão robusta de Poisson. A prevalência real de B. canis foi de 12,6% e de B. abortusfoi 22,8%. Os fatores associados com a soropositividade para B. canis foram idade maior que 10 anos (razão de prevalência; RP = 6,38; P = 0,024) e cães criados presos no quintal (RP = 5,20; P = 0,035) e para B. abortus foi não trocar a água dos animais todos os dias (RP = 1,48; P = 0,033). Conclui-se que a prevalência de B. canis e B. abortusem cães da região é alta, o que alerta para a necessidade de adoção de medidas de controle e prevenção, bem como são sugeridos maiores cuidados no manejo dos animais, sobretudo cães idosos.*
Palavras-chave: *Brucellacanis, Brucella abortus, saúde pública, epidemiologia, cães.*

INTRODUCTION

Canine brucellosis is an infectious zoonotic disease prevalent worldwide and is caused by bacteria of the genus *Brucella*. These bacteria affect mainly the reproductive tract of animals, causing infertility, abortion, placenta retention, weak offspring, and stillbirths in females, while causing orchitis and epididymitis in males (MÉLO et al., 2013; RODRIGUES et al., 2017). Its transmission

in dogs occurs by direct contact with vaginal fluids or infected semen (RODRIGUES et al., 2017), while in humans, the great majority of cases occur by consumption of contaminated dairy products or exposure to fluids and animal tissues of infected animals (ARENAS-GAMBOA et al., 2016).

In terms of public health, the close relationship between man and dog may lead to brucellosis transmission (DENTINGER et al., 2015). In man, the infection is usually neglected and underdiagnosed because of its diverse and unspecific symptoms, which make its diagnosis difficult (ROUSHAN et al., 2016).

In Brazil, the values of seroprevalence for *B. canis* in dogs are widely disparate depending on the locality, varying from 2 to 28% (RODRIGUES et al., 2017). Conversely, the serological surveys for *B. abortus* are rare. In the state of Paraíba, a few studies investigating the prevalence of *B. canis* in dogs have been conducted (ALVES et al., 2003; VASCONCELOS et al., 2008; FERNANDES et al., 2011), but only one study was conducted on *B. abortus* (AZEVEDO et al., 2003). In addition, there are no studies that show the occurrence of canine brucellosis in the Brejo Paraibano microregion, a place which has characteristics that may influence the occurrence of infection such as the presence of fragments of the Atlantic Forest, high rainfall, and a wide variety of fauna that may serve as natural reservoirs (ZHELUDKOV & TSIRELSON, 2010). Thus, this research aimed to determine the seroprevalence of *B. canis* and *B. abortus* in the canine population of the Brejo Paraibano microregion and identify factors associated with seropositivity.

MATERIALS AND METHODS

This study was conducted on dogs in the urban area of the Brejo Paraibano microregion, State of Paraíba, Brazilian Northeast. The region has a territorial area of 840.452 km^2, a population of 116,488 inhabitants, and is composed of eight municipalities: Alagoa Grande, Alagoa Nova, Areia, Bananeiras, Borborema, Matinhas, Pilões and Serraria.

To define the minimum number of animals to be sampled, a formula was used for simple random samples (THRUSFIELD, 2007) which considered the following parameters: an expected prevalence of 50% (maximization of sample), a confidence level of 95% (z = 1.96), and a sample error of 5%, resulting in a minimum sample size of 384 dogs. However, 386 dogs more than three months old, of both sexes, and varied breeds were used. The minimum number of dogs to be sampled in each municipality of the microregion was defined proportionally according to the number of inhabitants (Table 1).

The field work research conducted by a trained team by visiting the homes of the residents of the microregion. The dog owners were made aware of the study's objectives and, after agreeing to participate, signed informed consent forms. Blood collection was conducted between May to October 2017. The owners were made to answer a 35-item questionnaire in order to obtain data for analysis of factors associated with seroprevalence. Variables considered on the questionnaire were related to the dogs' information, owner, residence, and characteristics of animal management.

Table 1 - Number of residents, minimum number of dogs to be sampled, and animals positive for *Brucella canis* and *Brucella abortus* in each municipality of the BrejoParaibanomicroregion from May to October 2017.

Municipality	Residents[*]	Minimum number of dogs to be sampled	------------------*B. canis*-----------------		----------------*B. abortus*---------------	
			Sampled dogs	Positive dogs (%)	Sampled dogs	Positive dogs (%)
Alagoa Grande	28.479	94	100	4 (4.0)	96	29 (30.2)
Areia	23.829	78	71	11 (15.5)	59	12 (20.3)
Bananeiras	21.851	72	72	6 (8.3)	64	18 (28.1)
Alagoa Nova	19.681	65	67	4 (6.0)	65	9 (13.8)
Serraria	6.238	21	21	3 (14.3)	23	9 (39.1)
Pilões	6.978	23	21	2 (9.5)	24	6 (25.0)
Borborema	5.111	17	17	0 (0)	19	6 (31.6)
Matinhas	4.321	14	17	0 (0)	16	5 (31.2)
Total	116.488	384	386	30 (7.8)	366	94 (25.7)

[*]Source: Brazilian Institute of Geography and Statistics [IBGE].

Research of anti-*B.canis* antibodies was conducted using the agar gel immunodiffusion (AGID) technique through kits produced by the Institute of Technology of Paraná (TECPAR). The technique was conducted according to the manufacturer's recommendations using lipopolysaccharides and proteins antigens of *Brucella ovis*, sample Reo 198. To confirm, the same technique was applied in serum treated by 2-mercaptoetanol (AGID+2-ME) according to AZEVEDO et al. (2004). For the anti-*B.abortus* sp. antibodies research, the Buffered Acidified Antigen (BAA) technique was performed through the use of kits produced by TECPAR. The antigen consisted of an inactivated suspension of *B. abortus* sample 1119-3, stained by Rose Bengal, and diluted 8.0% in buffer solution pH 3.65 ± 0.05. Due to the mixture's pH, some unspecific agglutinins are inhibited, thus allowing an increase in test specificity.

Apparent and real prevalences were calculated according to NOORDHUIZEN et al. (1997). Apparent prevalence was obtained by dividing the number of seroreactive animals by the total number of animals, while real prevalence was estimated by adjusting the apparent prevalence considering sensitivity (52.94%) and specificity (98.7%) of the diagnosis using the *B. canis* protocol (KEID et al., 2009), as well as the diagnosis sensitivity (100%) and specificity (96.29%) for infection by *B. abortus* (TALUKDER et al., 2011) according to this formula:

$$RP = \frac{(AP + ESP - 1)}{(SEN + ESP - 1)}$$

Where,

RP = Real prevalence
AP = Apparent prevalence
ESP = Specificity
SEN = Sensitivity

To identify factors associated with seropositivity, a univariable analysis was initially conducted in which each independent variable was submitted to an association analysis with a dependent variable (seropositivity in the serological tests). Variables with $P \leq 0.2$ in the chi-square test were selected for multivariable analysis using robust Poisson regression. The collinearity between independent variables was verified by a correlation analysis. For the variables with strong collinearity (correlation coefficient > 0.9), one of two variables was excluded in the multiple agreement analysis with biological plausibility. To assess the adjustment of the regression model, the Pearson chi-square test was used. The level of significance adopted in the multiple analysis was 5%, and the analyses were conducted in R (R CORE TEAM, 2019) using the

"Epi" (CARSTENSEN et al., 2019), "Sandwich" (BERGER et al., 2017), and "lmtest" packages (ZEILEIS &HOTHORN, 2002).

RESULTS AND DISCUSSION

Of the 386 examined samples for *B. canis*, 99 were reactive in AGID, of which 30 were further positive in AGID+ME. This confirmed an apparent prevalence of 7.8% and real prevalence of 12.6%. Of the 366 examined samples for *B. abortus*, 94 were reactive in BAA, with anapparent prevalence of 25.7% and a real prevalence of 22.8%. Depending on locality, the prevalence of *B. canis* varied from 0 to 15.5%, while it varied from 13.8-39.1% in *B. abortus* (Table 1 and Figure 1).

The high real prevalence of *B. canis*and *B. abortus* in dogs of the Brejo Paraibano microregion is alarming due to the health risks posed to the public (DENTINGER et al., 2015). All of the cities in the microregion had seropositive dogs for *B. abortus*, showing that the agent is disseminated all over the region. These data should encourage adoption of control measures for preventing occurrence of the disease in dogs, slowing its propagation, and preventing it from being transmitted to human beings and other animal species. It should be highlighted that the characteristics of the studied region, including its high humidity and warm temperature for most of the year, may have facilitated the maintenance of bacteria in the environment.

In this study, BAA was used as an option to identify seroreactive dogs for *B. abortus* due to the technique's high sensitivity and specificity (TALUKDER et al., 2011). In addition, in bovines and small ruminants, BAA showed better performance than the tube agglutination test (CHACHRA et al., 2009; SADHU et al., 2015). Moreover, due to its excellent sensitivity and specificity in detecting IgM, IgG, and IgA of infected patients with brucellosis, it has good applicability in humans (DÍAZ et al., 2011).

It is possible that the high prevalence of *B. abortus* observed in dogs of the Brejo Paraibano microregionis also related to the presence of infected bovines in the region. In an official epidemiological study for bovine brucellosis in the state of Paraíba (CLEMENTINO et al., 2016), it was shown that the mesoregion of Agreste and Mata Paraibana, which included the study area, had the greatest prevalence of seroreactive bovines for *B. abortus*. Hence, taking into consideration the mode of transmission of the disease, it is possible that dogs in the region may have comein contact with fetal or placenta remains of infected
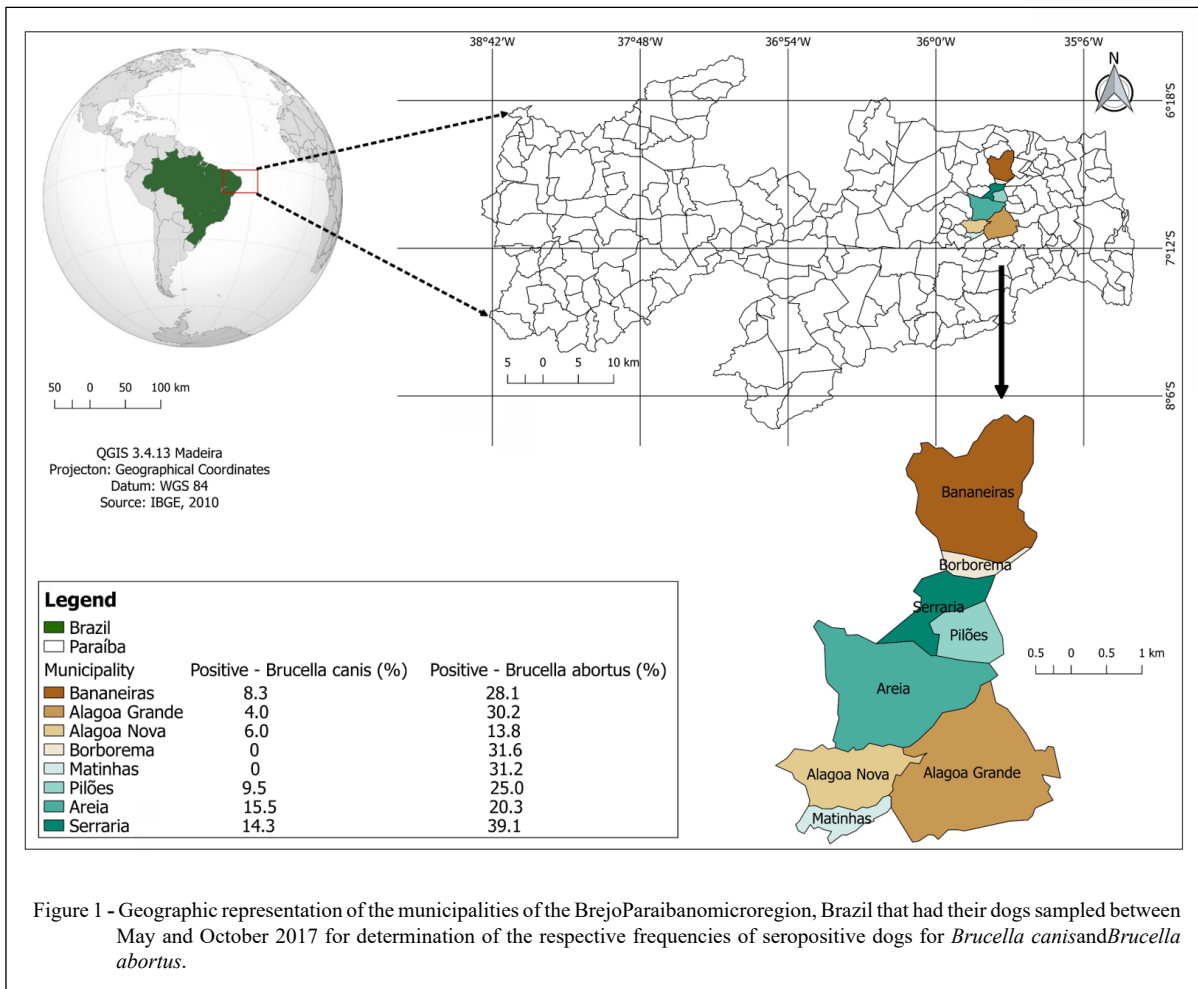
Figure 1 - Geographic representation of the municipalities of the BrejoParaibanomicroregion, Brazil that had their dogs sampled between May and October 2017 for determination of the respective frequencies of seropositive dogs for *Brucella canis*and*Brucella abortus*.

bovines, or may have consumed milk products or raw meat of bovines infected by the pathogen.

Note that the region has several Atlantic Forest fragments near urban areas and is composed of great fauna and flora biodiversity, increasing the chances of domestic dogs having contact with wild animals that could act as a bacterial reservoir. In a study conducted by AZEVEDO et al. (2010), 26.6% of hoary foxes (*Pseudalopexvetulus*) were seroreactive for *B. abortus* in BAA in the semiarid region of Paraíba.

In table 2, the factors associated with prevalence of *B. canis* and *B. abortus* that were selected in the univariable analysis ($P \leq 0.2$) are shown. For *B. canis*, the following variables were selected: type of rearing ($P = 0.006$), age ($P = 0.042$), animal baths ($P = 0.096$) and severe infestation by fleas ($P = 0.052$). For *B. abortus*, breed ($P = 0.105$), age ($P = 0.194$) and frequency of water exchange in the animal's bowel ($P = 0.123$) were selected. After robust Poisson regression analysis, two factors were confirmed to be associated with serore activity for *B. canis* and one for *B. abortus*. These were an age greater than 10 years old (prevalence ratio - PR = 6.38; CI 95% = 1.28 – 31.86; $P = 0.024$) and dogs raised in the backyard (PR = 5.20; CI 95% = 1.12 – 24.12; $P = 0.035$) for *B. canis*, while it was not changing the animal's water every day (PR = 1.48; CI 95% = 1.03 – 2.13; $P = 0.033$) for *B. abortus*. Both models for *B. canis* (Pearson's chi-squares = 333.23; degrees of freedom (df) = 377; value/df =0.884) and *B. abortus* (Pearson's chi-squared = 274.48; df = 359; value/df =0.765) had a good fit.

In the present research, robust Poisson regression was used to estimate PR, and it was observed that dogs older than 10 years old had more chances of being seropositive (PR = 6.38). This finding may be related to greater exposure time to

Table 2 - Univariable analysis of factors associated (*P*<0.20) with seroprevalence of *Brucella canis* and *Brucella abortus* in dogs of the Brejo Paraibano micro region.

Variable	Categories	Total number of dogs	Positive dogs (%)	*P*
--*Brucella canis*--				
Breeding	At home without street access	58	10 (17.2)	0.006
	At home with street access	252	18 (7.1)	
	Loose	76	2 (2.6)	
Age	Up to 1 year	83	3 (3.6)	
	2 – 10 years	290	24 (8.3)	0.042
	Above 10 years	13	3 (23.1)	
Do the animals take baths?	Yes	335	29 (8.7)	0.096
	No	51	1.0 (2.0)	
Severe flea infestation	No	372	27 (7.3)	0.052
	Yes	14	3 (21.4)	
--*Brucella abortus*--				
Breed	Without defined breed	304	73 (24.0)	0.105
	With defined breed	62	21 (33.9)	
Age	Up to 1 year	80	18 (22.5)	
	2 – 10 years	273	70 (25.6)	0.194
	Above 10 years	13	6 (46.2)	
Frequency of water change in the animal's bowl	Daily	282	67 (23.8)	0.123
	Does not change every day	84	27 (32.1)	

the agent when compared to younger animals. However, it is noteworthy that 87% of the sampled adult animals in this study were not neutered, which could be an associated factor. Thus, venereal disease transmission remains to be an important mode of transmission due to contact with vaginal fluids (KEID et al., 2007), urine and semen of infected animals (VOLKWEIS et al., 2018). The older the animal, the greater the chances of mating. and wider the variety of sexual partners; which consequently, leads to more chances of being possibly contaminated with the etiological agent.

Regarding the way the animals were raised, it was observed that dogs raised stuck in a backyard had more chances of being seropositive for *B. canis* (PR = 5.20). However, it must be highlighted that these animals did not have access to the street and had little contact with humans due to high aggressiveness, which may be an associated factor. Since a large portion of backyards in the houses of the region had only fences, these animals may have been able to have sexual contact with other dogs possibly entering these backyards, thus exposing them to a contracting a variety of infectious diseases.

Not changing the animals' water daily also increased the chance of seropositivity for *B. abortus* (PR = 1.48). FERNANDES et al. (2011) verified that failure to adequately clean the environment of the animals was associated with seropositivity. This may be related to contamination of water by fluids of the reproductive tract possessing the infectious agent, which consequently leads to maintenance of bacteria after failing to change the water. Since these bacteria are known to survive for months in potable water, this reinforces the importance of changing the water in animals' bowls daily.

CONCLUSION

The current study's results indicated a high prevalence of dogs seropositive for *B. canis* and *B. abortus* in the Brejo Paraibano microregion, which should encourage urgent implementation of control and prevention measures for this species. Based on the association analysis, proper management of animals, especially of older dogs, must be encouraged.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the study's design and its conduct, as well as the preparation of this article, which all authors also revised and approved.

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