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MICROBIOLOGY

Anti-*Brucella* spp. antibodies in working equids in a semiarid region of Northeastern Brazil

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ABSTRACT: The present study described the prevalence and factors associated with the seropositivity for anti-*Brucella* spp. antibodies in working equids in the semiarid regionofnortheastern Brazil. Blood samples were collected from 322 equids (76 horses, 155 mules and 91 donkeys). The Rose Bengal plate test (RBPT) was used as a screening method. Samples that were reactive in the RBPT were tested for confirmation using 2-mercaptoethanol (2-ME) and the standard tube agglutination test (STAT). Overall, 7.1% (23/322) of the samples were positive according to the RBPT. After confirmatory tests, the prevalence of brucellosis was found to be 6.5% (21/322). Among these cases, 33.3% (7/21) were horses, 42.8% (9/21) were mules and 23.9% (5/21) were donkeys. In the multivariate analysis, age over 10 years was considered to be a factor associated with the seropositivity (OR: 17.17; 95% CI: 2.582-164.7, P = 0.006) for anti-*Brucella* spp. antibodies. The significant prevalence of anti-*Brucella* spp. antibodies found in working equids and the wide distribution of positive animals demonstrates a worrying epidemiological situation regarding brucellosis in the study region. Age over 10 years was a risk factor associated with the seropositivity for anti-*Brucella* spp. antibodies in animals.
Key words: donkeys, brucellosis, horses, mules, one health.

Anticorpos anti-*Brucella* spp. em equídeos de tração em uma região semiárida do Nordeste do Brasil

RESUMO: O objetivo do presente estudo foi descrever a prevalência e os fatores associados à infecção por *Brucella*spp. em equídeos de trabalho na região semiárida do Nordeste do Brasil. Amostras de sangue foram coletadas de 322 equídeos (76 equinos, 155 muares e 91 asininos). O teste de placa Rosa Bengala (RBPT) foi usado como método de triagem. As amostras que foram reativas no RBPT foram enviadas para confirmação usando 2-mercaptoetanol (2-ME) e o teste padrão de aglutinação em tubo (STAT). Ao todo, 7,1% (23/322) das amostras foram positivas segundo o RBPT. Após testes confirmatórios, a prevalência de brucelose foi de 6,5% (21/322). Destes casos, 33,3% (7/21) foram equinos, 42,8% (9/21) muares e 23,9% (5/21) asininos. Na análise multivariada, idade acima de 10 anos foi considerada fator associado à infecção (OR: 17,17; 95% CI: 2,582-164,7, P = 0.006) por *Brucella*s pp. A prevalência significativa de anticorpos anti-*Brucella* spp. encontrados em equídeos de trabalho e a ampla distribuição de animais positivos demonstra uma situação epidemiológica preocupante em relação à brucelose na região de estudo. A idade superior a 10 anos foi um fator de risco associado à infecção em animais.
Palavras-chave: asininos, brucelose, equinos, muares, saúde única.

INTRODUCTION

Brucellosis is a disease with zoonotic potential caused by bacteria of the genus *Brucella*, with greater prevalence in developing countries within tropical regions (MCDERMOTT et al., 2013; NJOGA et al., 2018). It is listed in the Terrestrial Animal Health Code, World Organization for Animal Health (WOAH, 2022), and is considered a disease of high socioeconomic and public health impact. This has consequences for the marketing of animals and their products. Brazil has a national program for controlling and eradicating brucellosis and tuberculosis (Programa Nacional para o Controle e Erradicação de Brucelose e Tuberculose, PNCEBT) (BRASIL, 2020) that is aimed only at cattle and buffaloes. However, there are no specific technical regulations for diagnosis and control in relation to other animal species.

Brucellosis has high transmissibility and several types of host can be sources of infection, through contact with secretions and consumption of unpasteurized products (MEGID, 2016). In horses, brucellosis is mainly caused by *Brucella abortus*, which causes chronic disease, with joint and synovial inflammation and reproductive disorders (PINHO et al., 2014; MEGID, 2016). Once infected, equids acts as reservoirs and secondary hosts for the spread of brucellosis, as they may make long daily journeys while maintaining contact with humans and other

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species (SANTOS et al., 2012; SUNG & YOO, 2014; LOTFI et al., 2022)

In rural areas, equids play an important economic and social role, through performing haulage work. The health of these animals and their physical performance are crucial for income generation, since these animals are the basis of financial support for countless families (TAVARES et al., 2015). However, brucellosis can cause joint and reproductive problems, thus directly causing reductions in the work capacity of equids (DORNELES et al., 2013).

There are only a few studies on the prevalence of brucellosis among working equids. In Brazil, its prevalence has been found to range from 0% to 5.7% (DORNELES et al., 2013; PINHO et al., 2014; ROCHA et al., 2019; RESENDE et al., 2022). In Nigeria, prevalence of 3% has been reported (NJOGA et al., 2018), while in Pakistan, the prevalence was found to reach 20.1% (GUL et al., 2013).

Considering the few studies on these animals and the importance of this disease, especially in the context of one health, this study determined the prevalence and factors associated with the seropositivity for anti-*Brucella* spp. antibodies among working equids in the semiarid region of northeastern Brazil.

To determine the minimum number of animals to be sampled, simple random sampling was used:

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

n = sampling number
Z = normal distribution value for the 95% confidence level
P = expected prevalence of 6.5% (ANTUNES et al., 2013)
d = 3% sampling error

To perform adjustments for finite populations, the following formula was applied:

$$najus = \frac{N \times n}{N + n}$$

n_{ajus} = adjusted sample size
N = total population size
n = initial sample size

The adjustment of the population sample size took into account the total population of equids in the state of Paraíba, which was taken to be 98,584 animals (IBGE; available at https://cidades.ibge.gov.br/brasil/pb/pesquisa/24/27745). Thus, the minimum number of animals required for participation in the study was 259. However, 322 samples were collected.

From July to December 2020, blood samples were collected from working equids, on the basis of convenience, in urban areas of 16 municipalities of the state of Paraíba, northeastern Brazil (Figure 1). These equids comprised 76 horses, 155 mules and 91 donkeys. They were at least one year of age and were selected regardless of breed and sex, as long as they were in traction activity in urban areas, with the owner.

External jugular venipuncture was aseptically accessed to collect 5 ml of blood from each working equid. The blood samples were placed in test tubes, identified and sent to the Laboratory of Immunology and Infectious Diseases (LIID) of the Federal Institute of Paraíba – IFPB. The samples were centrifuged at 4000 rpm for 15 minutes to obtain serum, which was then stored at -20 °C for serological tests for brucellosis to be performed.

Screening and confirmatory tests were carried out in accordance with Normative Instruction No. 10 of March 3, 2017, of the Agricultural Protection Department (BRASIL, 2017), which regulates the PNCEBT.

At LIID, the Rose Bengal plate test (RBPT) test was performed (ALTON et al, 1988), which presents high sensitivity, but low specificity, so, false positive can occur due to cross-reaction with antibodies of other pathogens (EHIZIBOLO et al., 2011; SIKDER et al., 2012). Samples that were found to be reactive in the RBPT were then sent to the Specialized Veterinary Research Institute (SVRI), in Belo Horizonte, Minas Gerais, for confirmatory tests using 2-mercaptoethanol (2-ME) and the standard tube agglutination test (STAT), high sensitivity and specificity (MEMISH et al., 2002), performed simultaneously (ALTON et al., 1988).

An epidemiological questionnaire was applied to the owners of these working equids, to collect information relating to the species (horse, donkey or mule), sex, breed and age. Data relating to environmental management, contact with other domestic animals (other equids, cattle, sheep, pigs, poultry, dogs and cats) and wild animals were also obtained, along with data about food management, considering the type of food and food storage. Lastly, data were obtained regarding reproductive and health characteristics, such as occurrences of abortions, the number of deliveries, deworming (frequency and active ingredient of the deworming agent), vaccination and present or past illnesses.

Risk factors associated with the seropositivity for anti-*Brucella* spp. antibodies were

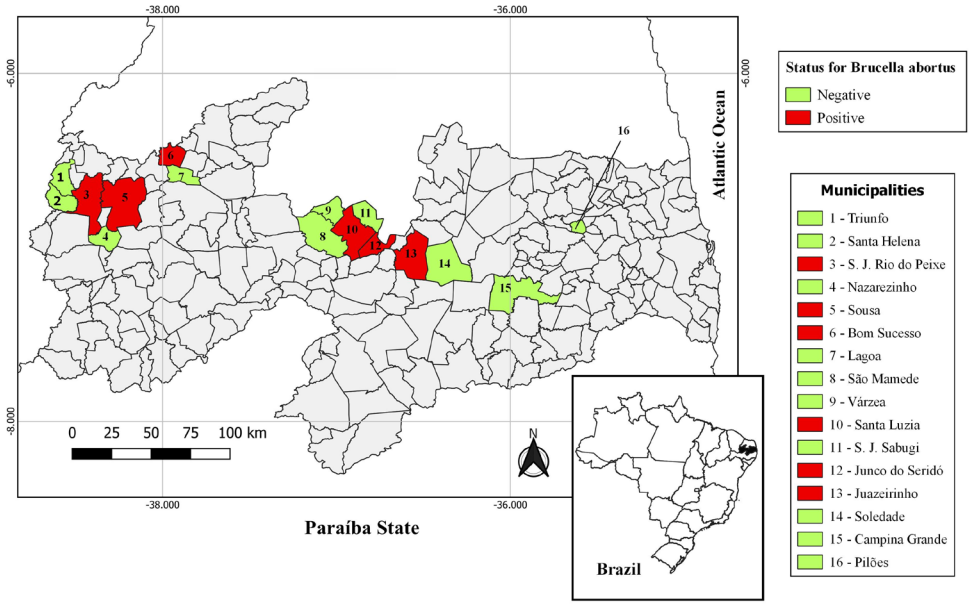

Figure 1 - Map showing the distribution of anti-*Brucella* spp. antibodies in seropositive and seronegative working equids among municipalities in the state of Paraíba, Northeastern Brazil.

evaluated through the data from epidemiological questionnaires, in two stages: univariate analysis and multivariate analysis. In the univariate analysis, each independent variable was submitted to an association analysis with the dependent variable(serological condition – positive or negative), and those that presented a p-value ≤ 0.2, according to the chi-square test or Fisher's exact test (ZAR, 1999), were selected for multivariate analysis, using multiple logistic regression (HOSMER & LEMESHOW, 2000). The significance level adopted in the multiple analysis was 5%.The final model was adjusted using the coefficient of Hosmer and Lemeshow to best fit the value to P ≥ 0.05. The collinearity of the independent variables was determined by correlation analysis, and when the correlation coefficient was <0.9, one of the variables was eliminated according to biological plausibility (DOHOO et al. 1997). The results were analyzed in the GraphPad Prism 9.5 software.

Among the animals evaluated in this study, it was observed that 7.1% (23/322) were positive in the RBPT screening test. However, only 6.5% (21/322; 95% CI: 5.3%-9.4%) were positive in both confirmatory tests (2-ME and STAT).

Among the 16 municipalities in the state of Paraíba that were visited, six had positive animals (Table 1). In these, positivity ranged from 5.7% (2/35) in Bom Sucesso to 33.3% (1/3) in Juazeirinho.

The prevalence among horses (9.2%; 7/76; 95% CI: 7.8% -11.3%), mules (5.8%; 9/155; 95% CI: 4.1% - 8.3%) and donkeys (5.5%; 5/91; 95% CI: 4.4% -7.1%) did not statistically differ (P > 0.2) (Table 2). Regarding the factors associated with *Brucella* spp. seropositivity, only the variables of age and contact with cats were selected for the multiple logistic regression (P ≤ 0.2). In the multivariate analysis, age ≥ 10 years was considered to be a factor associated with the seropositivity (OR: 17.17; 95% CI: 2.582-164.7, P = 0.006).

In this study, it was observed that traction equids, regardless of the species (horses, donkeys and mules), could be infected by *Brucella* spp. in the State of Paraíba, Northeastern Brazil. This suggested the importance of equids in the epidemiology of Brucellosis, as well as the need for health policies for these species, since they are in direct contact with humans and other animals.

In Brazil, there is no specific regulation for controlling brucellosis in equids. In the classification established through the PNCEBT for cattle, the state of Paraíba is included in class B, with a prevalence of outbreaks ranging from

Table 1 - Numbers of working equids from which blood samples were collected and percentages of seropositivity for anti-*Brucella* spp. antibodies according to municipalities in the state of Paraíba, Brazil.

Municipalities	----------Samples collected----------	----------Positive (%)----------
Bom Sucesso	35	2 (5.7)
Juazeirinho	3	1 (33.3)
Junco do Seridó	5	1 (20)
Lagoa	18	0
Nazarezinho	7	0
Pilões	9	0
Santa Helena	13	0
Santa Luzia	14	1 (7.1)
São João do Rio do Peixe	62	5 (8.1)
São José da Mata	3	0
São José do Sabugi	15	0
São Mamede	6	0
Soledade	3	0
Sousa	109	11 (10.1)
Triunfo	15	0
Várzea	6	0
Total	322	21 (6.5)

≥ 2% to < 5% (BRASIL, 2020). In the present study, the seroprevalence of 6.5% (21/322) among equids in the study region was higher than that of the classification used for cattle. In addition, wide distribution of the seropositivity was observed, such that 37.5% (6/16) of the municipalities visited had positive animals. These results denoted a situation of concern regarding possible increases in the levels of infection among other animals and among humans. Similar results were reported by RESENDE et al. (2022) among horses in the Brazilian Amazon region; they stated that monitoring the occurrence rate of brucellosis is extremely important since this disease affects the herds both economically and zootechnically, in addition to its high zoonotic potential.

A false-positive diagnosis was observed in two samples, after carrying out the 2-ME and STAT confirmatory tests. The RBPT, due to its simplicity, low cost and convenience is an important screening test. However, confirmatory methods with high sensitivity (100%) and specificity (95.6%) (MEMISH et al., 2002), cannot be dispensed to guarantee diagnostic security.

Equids can take on the role of sentinels in the epidemiology of some zoonoses (HACK et al., 2018). These are animals that commonly come into contact with cattle, which are the main hosts of *Brucella* spp. Horses are natural hosts; although, the infection is latent or subclinical, they are important for the epidemiology of the disease as a reservoir or secondary host of the bacteria. (LOTFI et al., 2022). In equids, the clinical form of the disease is characterized by lesions resulting from inflammation of the supra-atlantal bursa and associated connective tissue, which lead to suppuration and fistulation of the affected parts of the body (SILVA et al., 2001; IHEDIOHA & AGINA, 2014). The disease also gives rise to abortions, birth of weak foals, retained placenta, lameness due to polyarthritis, carpal bursitis, orchitis and epididymitis in stallions (TIJJANI et al., 2017).

Age over 10 years was considered to be a factor associated with the seropositivity for anti-*Brucella* spp. antibodies, similar to what was previously observed in the southeastern region of Brazil (JUNQUEIRA et al., 2015). In Pakistan, the lower prevalence observed among young animals was explained by the fact that younger animals are able to harbor *Brucella* spp. without expressing detectable antibodies until their first parturition or abortion, and for as long as they are not exposed to some physical or physiological stress (WADOOD et al., 2009). In addition, exposure of working equids to long distances, dynamics of varied paths and contacts with other species, including ruminants, may make them more susceptible to infection by *Brucella* spp. Thus, older animals

Table 2 - Univariate and multivariate analyses on risk factors associated with seropositivity for *Brucella* spp. among working equids in the semiarid region of the state of Paraíba, Brazil.

Variable / category	Univariate analysis			Multivariate analysis		
	Number of equids	Number of positive equids (%)	P	OR	95% CI	P
Species						
Horses	76	7 (9.2)	0.551			
Donkeys	91	5 (5.5)				
Mules	155	9 (5.8)				
Sex						
Male	138	8 (5.8)	0.820			
Female	184	13 (7.1)				
Age						
Up to 4 years	72	2 (2.8)	< 0.001*	Ref.		
5 to 9 years	129	7 (5.4)		3.79	0.78-28.12	0.127
≥ 10 years	120	12 (10.0)		17.17	2.58-164.7	0.006
Food						
Pasture	144	11 (7.6)	0.537			
Pasture + Corn	140	9 (6.4)				
Pasture + Commercial food	38	1 (2.6)				
Contact with dogs						
Yes	237	17 (7.2)	0.609			
No	85	4 (4.7)				
Contact with cats						
Yes	274	21 (7.7)	0.053*	0.97	0.24-5.12	0.969
No	48	0 (0)		Ref.		
Contact with ruminants						
Yes	169	12 (7.1)	0.801			
No	153	9 (5.9)				
Working time						
Up to 3 years	282	19 (6.7)	0.706			
4 to 8 years	40	2 (5)				

OR: Odds ratio; CI: Confidence interval; Ref.: Reference value.
*Variables that presented P ≤ 0.20 according to the chi-square test or Fisher's exact test.

tend to have been exposed for longer periods than younger animals.

The notable prevalence of anti-*Brucella* spp. antibodies found in working equids and the wide distribution of municipalities with positive animals demonstrates a worrying epidemiological situation for brucellosis in the state of Paraíba. Age over 10 years was a risk factor associated with the seropositivity for anti-*Brucella* spp. antibodies among these animals.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Ethics Committee for Use of Animals of Instituto Federal da Paraíba, Sousa campus, under approval number: 23000.000665.2020-71.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding 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

Conceptualization: PWLC, TFF and VLRV. Data acquisition: PWLC, CSMO and AS. Design of methodology and data analysis: TFF and VLRV. PWLC, FBVA and VLRV prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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