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Occurrence of anti-*Brucella abortus* and anti-*Leptospira* spp. antibodies in buffaloes from Paraíba state, Northeastern Brazil

Ocorrência de anticorpos anti-*Brucella abortus* e anti-*Leptospira* spp. em búfalos da Paraíba, Nordeste do Brasil

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Abstract

The objective of this study was to determine the frequency of animals that tested positive for brucellosis and leptospirosis and the risk factors for these diseases in the State of Paraíba. A total of 136 buffaloes from 14 herds were examined. For brucellosis, we used the buffered acidified plate antigen (BAPA) test as screening method and 2-mercaptoethanol as confirmatory test. For leptospirosis, we conducted a microscopic agglutination test (MAT), with a cut-off point of 1:100. Of the animals examined, two [1.5%; 95% CI = 0.4%–5.2%] were positive for brucellosis, and 38 (27.9%; 95% CI = 21.1%–36.0%) were positive for leptospirosis. The brucellosis-positive animals were from two (14.3%) herds, while nine (64.3%) herds had leptospirosis-seropositive animals. The more frequent *Leptospira spp*. serotypes were Bratislava, Pomona, and Canicola. We concluded that leptospirosis was widespread in buffaloes in the state of Paraíba and suggested that breeding alongside horses and pigs might be an important factor in the spread of leptospirosis-positive animals. The presence of brucellosis-positive animals indicated the possibility of negative-impacting measures on disease control in bovines, and it is therefore recommended that greater attention be given to these animals for brucellosis control.

Key words: *Brucella abortus*, buffalo, *Leptospira spp*, Northeast Brazil, seroepidemiology

Resumo

O objetivo do trabalho foi determinar a frequência de animais reagentes e os fatores de risco para brucelose e leptospirose em búfalos do Estado da Paraíba. Foram utilizados 136 búfalos oriundos de 14 propriedades. Para o diagnóstico da brucelose empregou-se como teste de triagem o antígeno acidificado tamponado (AAT) e o teste do 2-mercaptoetanol (2-ME) como prova confirmatória. Para leptospirose foi realizado o teste de soroaglutinação microscópica (SAM), com ponto de corte 1:100. Dos 136 animais examinados dois (1,5%; IC 95% = 0,4% - 5,2%) foram positivos para brucelose e 38 (27,9%; IC 95%

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= 21,1% - 36,0%) para leptospirose. Os animais positivos para brucelose foram procedentes de duas (14,3%) propriedades, enquanto para leptospirose nove (64,3%) propriedades apresentaram animais soropositivos. Os sorovares de *Leptospira* spp. mais frequentes foram Bratislava, Pomona e Canicola. Conclui-se que a leptospirose encontra-se disseminada em búfalos do Estado da Paraíba, e sugere-se que a criação consorciada com equinos e suínos pode ser um fator importante na ocorrência de animais positivos. A presença de animais positivos para brucelose indica a possibilidade de impacto negativo nas ações de controle da doença em bovinos, e dessa forma recomenda-se que maior atenção seja dada a esses animais do ponto de vista do controle da brucelose.
Palavras-chave: *Brucella abortus*, búfalo, *Leptospira* spp., Nordeste do Brasil, soroepidemiologia

Introduction

Renowned for their hardiness, tolerance, and resistance to infectious and parasitic diseases, buffaloes are more adapted than cattle to adverse environmental conditions. Because they are versatile animals, they have become a great alternative to the national livestock, as they have triple the ability (milk production, meat production, and traction) and are easily adapted to harsh environments, such as floodplains, coastal areas, mountains, and plains (TONHATI et al., 2011).

Currently, Brazil has a prominent position in buffalo ranching with more than a million heads, and the Northeast has around 122,000 animals. In the State of Paraíba, the number of effective buffalo is approximately 933 animals (BRASIL, 2012).

The increased productivity and technology in buffalo ranching favors the introduction of infectious diseases into the herd due to management changes, increased animal density, and confinement (LEITE; BASTIANETTO, 2009). In this context, brucellosis and leptospirosis are important because they are zoonotic diseases that are responsible for economic losses in buffalo-ranching operations due to reproductive problems.

Brucellosis is a zoonotic disease that is caused by *Brucella abortus*, which affects the reproductive system (ACHA; SZYFRES, 2001; PAULIN; FERREIRA NETO, 2008). The infection is widespread in developing countries (GHODASARA et al., 2010) and is responsible for economic losses resulting from abortions, low reproductive rates, loss of reputations of properties, and restrictions on the commercialization of animals and animal products coming from areas where the disease occurs (PAULIN; FERREIRA NETO, 2008). It is believed that brucellosis is responsible for a 25% reduction in the production of milk and meat and a 15% reduction in the production of calves (PAULIN, 2006). The most common form of transmission is the ingestion of contaminated water, pasture, and fodder. Another frequent form of transmission is direct contact with infected aborted fetuses and newborn calves (ACHA; SZYFRES, 2001; PAULIN; FERREIRA NETO, 2008).

Leptospirosis, which is the zoonosis with the largest worldwide distribution, is present on all continents. Leptospirosis is classified into 13 pathogenic genome species with over 260 serotypes and six saprophytes containing more than 60 serotypes. In general, each serotype has a more adapted host, but it can infect other hosts (ADLER; MOCTEZUMA, 2010). The transmission of the disease is through contact with contaminated urine, water, soil, and fomites. The presence of rodents is considered a risk factor because they behave like healthy kidney carriers of Leptospira, eliminating them into the environment and contaminating soil and water (ADLER; MOCTEZUMA, 2010). In Latin America, this disease is endemic and causes huge economic losses (SCHMITD et al., 2002).

Buffaloes are affected by leptospirosis similarly to cattle. The main consequence of systemic infection with leptospirosis is abortion. The abortions usually

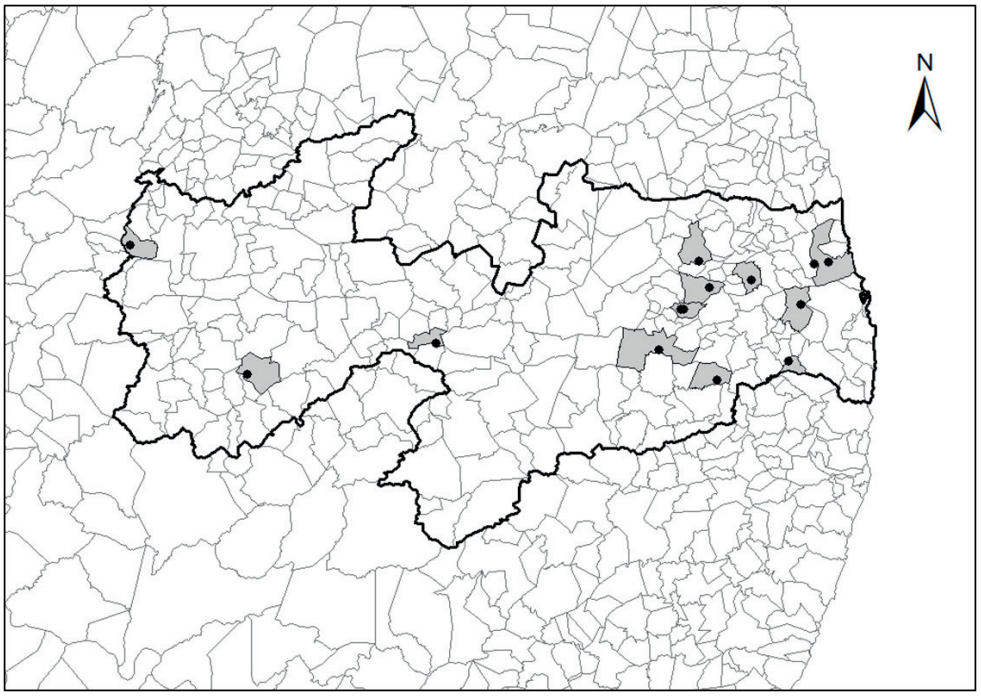
occur in the third trimester of gestation and cause fetal death, with or without placental degeneration, which is followed by removal of the fetus weeks after infection (FAINE et al., 1999).

In Brazil, in recent years, the frequency of seropositivity for buffalo leptospirosis has been reported to range from 37.7% to 80% (LANGONI et al., 1999; VIANA et al., 2009); for brucellosis, this variation was from 5.18% to 37.5% (BASTIANETTO et al., 2005; CHAVES et al., 2012). In the absence of studies on buffalo leptospirosis and brucellosis in the State of Paraíba, this study aimed to determine the occurrence of anti-*Leptospira spp*. and anti-*Brucella abortus* antibodies in buffaloes in the State of Paraíba and to identify risk factors that were associated with the infections.

Materials and Methods

This study was conducted on 14 buffalo-ranching herds in the State of Paraíba in Alagoa Nova, Areia, Campina Grande, Guarabira, Juripiranga, Santa Helena, Sapé, Rio Tinto, Santana of Garrotes, Itatuba, Solânea, and Cacimbas (Figure 1). According to the Secretary of Agricultural Development, Livestock, and Fisheries in the State of Paraíba (SDALF), the state has 17 herds and an effective number of 872 animals.

Figure 1. Geographical distribution of counties and herds used in the State of Paraíba.



The studied population consisted of female buffaloes that were used for beef and dairy purposes, were crossbreeds or Murrah breeds, and were 24 months old or older. To calculate the number of animals to sample, we used the following formula for simple random sampling:

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

Where,

n = number of animals to sample

Z = value of the normal distribution for a

confidence level of 95%

P = expected prevalence of 50% (for maximization of the sample)

D = error of 10%

According to the sample calculation, the study needed 96 animals. In total, we selected 136 female buffaloes aged ≥24 months from 14 herds whose owners agreed to participate. We selected all existing females in the herd at the time of the visit to the herd. Blood samples were collected from November 2012 to July 2013 through jugular venipuncture after antisepsis with the aid of a disposable 40 × 12-mm needle and stored in sterile test tubes, which remained inclined and at rest in order to facilitate clot retraction. The samples were centrifuged for 15 min, and the serum that was obtained was transferred to microtubes and stored at -20°C.

During sample collection, we conducted an epidemiological survey to obtain data to be used in the risk factor analysis. The variables and categories used were the following: type of farming (intensive, semi-intensive, or extensive), type of operation (meat, milk, or mixed), type of milking (manual, mechanical milking, or mechanical with milking parlor), number of milkings per day (not milking, 1 time a day, or two times a day), predominant breed, other species on the property (cattle, horses, goats/sheep, pigs, poultry, dogs, or cats), wild species on the property, occurrence of abortions in the last 12 months, the presence of rodents, rodent control, feeding on native pasture, water supply (drinking fountains, watering, rivers, lakes, streams, or springs), previous diagnosis of brucellosis, vaccines administered against brucellosis, acquisition of animals, rental pastures, presence of wetlands, presence of a calving paddock, separation of young adult animals, milk cooling, and veterinary care.

In order to perform the test of buffalo brucellosis, we initially used the buffered acidified plate antigen (BAPA) test as screening, and the positive sera underwent a confirmatory test with 2-mercaptoethanol (2-ME) (BRASIL, 2006). Alongside the 2-ME test, we performed the standard tube agglutination test (STAT).

The leptospirosis test was conducted by the microscopic agglutination test (MAT) according to the methods of Galton et al. (1965) and Cole et al. (1973), and as live antigens the following 22 pathogenic and two saprophytes serotypes were used: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola Whitcombi, Cynopteri, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, Andamana, Patoc, and Sentot. The sera were screened at a dilution of 1:100, and those with 50% or more agglutination were titrated by taking a geometric series dilution with a ratio of two. The serum titer was the reciprocal of the highest positive dilution. The antigens were examined with dark-field microscopy prior to the tests in order to verify mobility and the presence of autoagglutination or contaminants. In cases of coagglutination, we considered the serotype corresponding to the serum that had the highest titer.

The risk factor analysis was performed in two steps: univariate analysis and multivariate analysis. In the univariate analysis, the variables with $p \leq 0.2$ on the chi-square test or Fisher's exact test (ZAR, 1999) were selected and used in the multivariable analysis that consisted of a multiple logistic regression (HOSMER; LEMESHOW, 2000). The significance level in the multiple analyses was 5%. All analyses were performed with SPSS 20.0 for Windows.

Results and Discussion

Of the 14 herds studied, two (14.3%) had one brucellosis-positive animal each and nine (64.3%) had at least one animal that was seropositive for leptospirosis (Table 1). Of the 136 animals examined, 14 (10.3%) were seropositive for brucellosis on the BAPA test and two [1.5%; 95% CI = 0.4%–5.2%] were seropositive in the 2-ME test, with titers of 25

and 200. For leptospirosis, 38 (27.9%; 95% CI = 21.1%–36.0%) of the 136 samples were seropositive in the MAT, with titers that ranged from 100 to 3,200. The most common serotypes (Table 2) were Bratislava (11%), Pomona (8.8%), and Canicola (5.9%). In addition, reactions were observed for the Patoc (0.74%), Wolffi (0.74%), and Cynopteri (0.74%) serotypes.

Table 1. Frequency of reactant buffaloes to leptospirosis and brucellosis in the State of Paraíba, according to counties and herds, in the period from November 2012 to July 2013.

County/Herds	Total no. of animals	*Leptospira* spp.		*Brucella abortus*	
		Nº	%	Nº	%
Santa Helena/ Herd 1	15	4	26.6	0	0
Cacimbas/ Herd 2	2	0	0,0	0	0
Rio Tinto / Herd 3	19	8	42.1	0	0
Sapé/ Herd 4	12	1	8.3	0	0
Itatuba/ Herd 5	20	8	40	1	5
Guarabira/ Herd 6	5	3	60	0	0
Campina Grande/ Herd 7	23	8	34.7	1	4.3
Solânea/ Herd 8	3	0	0	0	0
Santana dos Garrotes/ Herd 9	4	2	50	0	0
Juripiranga/ Herd 10	1	0	0	0	0
Alagoa Nova/ Herd 11	5	0	0	0	0
Alagoa Nova/ Herd 12	10	3	30	0	0
Rio Tinto/ Herd 13	13	1	7.6	0	0
Areia/ Herd 14	4	0	0	0	0
Total	136	38	27.9	2	1.5

Table 2. Most frequent *Leptospira* spp. serovars in buffaloes in the State of Paraíba, in the period fom November 2012 to July 2013.

Serovar	Proportion of reactant animals	Frequency (%)
Bratislava	15/136	11
Pomona	12/136	8.82
Canicola	8/136	5.88
Patoc	1/136	0.74
Wolffi	1/136	0.74
Cynopteri	1/136	0.74

For the risk factors, we found no association between any variable and seropositivity for brucellosis or leptospirosis in the univariate analysis, which precluded the need to perform the multivariate analysis. Although only two animals were positive for brucellosis, this result had considerable importance because the infection is the target of official control and the presence of seropositive animals can ensure the persistence of this important zoonotic agent in herds. Additionally, the consociated breeding of cattle and buffaloes was observed on some herds. Because of the possibility of transmission of brucellosis between the bovine and buffalo species, the chance of a negative

impact on the actions of the National Program for Control and Eradication of Bovine Brucellosis and Tuberculosis (NPCEBBT) should not be ruled out because these efforts are conducted mainly on cattle in the State of Paraíba. The two herds that were positive for brucellosis were located in the Agreste and Mata Paraibana mesoregions, which are locations that had a higher prevalence of bovine brucellosis compared to the Sertão and Borborema mesoregions in the epidemiological survey that was conducted as part of NPCEBBT in Paraíba (unpublished data).

Beyond the economic importance, another point to consider is that the presence of brucellosis-positive buffaloes involves the exposure of humans to the risk of infection due to the consumption of animal products, such as milk and dairy products. Borriello et al. (2006) reported a potential risk for humans after the consumption of milk and dairy products from buffaloes infected with *Brucella abortus*.

Chaves et al. (2012) in Maranhão and Calderón et al. (2010) in Colombia reported the prevalence of brucellosis in buffaloes as 5.18% and 3%, respectively. The low frequency (1.5%) of brucellosis that was found in this study may be due to the number of bovine brucellosis control actions that are conducted by the animal defense service of Paraíba, including the inspection of vaccinations and animal movement control.

For leptospirosis, the high frequency of herds that were positive and seropositive animals suggested that the agent is spread in buffalo herdsin the state. Langoni et al. (1999) evaluated 403 buffaloes from Vale da Ribeira in São Paulo, and they observed a prevalence of 37.7% for the Wolffi, Icterohaemorrhagiae, and Hardjo serotypes. Silva et al. (2009) examined 127 buffaloes from the northeastern Pará region and found a frequency of 67.72% seropositivity for the Hardjo, Grippotyphosa, and Pomona serotypes. The high frequency of positive tests for leptospirosis in buffaloes may reflect a variety of factors that influence the occurrence of the disease, such as the contact animal species, management used, existing serotypes in the region, climatic and environmental conditions, and opportunities for direct or indirect infection (HIGINO et al., 2012).

The Bratislava serotype, which is described as the most frequent, has the most adapted hosts, including horses and pigs (BOLIN, 1996). This may be associated with the contact of buffaloes with horses and pigs in breeding consortiums, which in fact was observed on some properties. From the point of view of leptospirosis control, the Bratislava serotype being the most frequent raises concerns because the anti-leptospirosis commercial vaccines for buffaloes includes antisera for the Hardjo, Wolffi, Pomona, Grippothyphosa, Icterohaemorrhagiae, and Canicola serotypes, and there is no cross-protection between serotypes.

The second most common serotype was Pomona, whose maintenance host is the pig (FAINE et al., 1999), which also suggests contact with these species in breeding consortiums. The Canicola serotype, which was named the third most frequent, is usually associated with dogs as a maintenance host (FAINE et al., 1999). Based on the epidemiological surveys, most of the properties had dogs present, and some of these animals were used in the management of buffalo. Thus, there is the possibility of transmission between species.

Conclusion

We concluded that leptospirosis was widespread in buffaloes in the State of Paraíba, and the results suggested that breeding consortiums with horses and pigs were an important factor in the occurrence of test-positive animals. The presence of animals that tested positive for brucellosis indicated the possibility of a negative impact on the disease control measures in bovines, and it is therefore recommended that greater attention be given to these animals from the point of view of brucellosis control.

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