Detection of brucellosis in water buffaloes in northern and northeastern Brazil

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ABSTRACT – The prevalence of brucellosis in buffaloes was evaluated by the Rose Bengal test (RBT) in 5170 water buffaloes from Maranhão state, Pará state and Marajó Island,
Brazil. The detection of buffaloes positive for brucellosis by RBT was 7.37% in Marajó Island, 8.45% in Pará state and 29.86% in Maranhão state. The locations with the highest prevalences were Santa Cruz do Arari, in Marajó Island (12.50%); Irixuna, in Pará state (30.25%); and Santa Inês, in Maranhão state (34.76%). After the Complement Fixation (CF) confirmatory test, only 7 animals remained positive in Marajó Island, and 22 remained positive in the state of Pará. None of the 66 animals that reacted positively in the RBT test in Maranhão reacted positively in the CF confirmatory test. The prevalence of brucellosis was significantly different (p<0.05) between the three regions evaluated. The high prevalence of *B. abortus* that was observed in animals, especially in the state of Maranhão, is worrisome for the health system for the control and eradication of bovine brucellosis. These results show the importance of brucellosis in buffaloes, thereby indicating the imminent need for a brucellosis control program specific to these animals in Brazil.

**KEYWORDS:** RBT; Buffaloes; Brazil; *Brucella abortus*.

**INTRODUCTION**

Brazil has approximately 1.3 million buffaloes, thus constituting the largest buffalo herd in the West [1]. Of these, 63% inhabit the northern region of the country, where the buffaloes are used for both the export of live animals and consumption of meat and milk. According to recent research conducted in Brazil, the buffalo herd showed a significant growth between 2010 and 2011, with an increase of 7.8% [1].

Bovine brucellosis is a bacterial disease caused by *Brucella abortus*. The disease is one of the major zoonotic diseases in the world [2]. This disease can be easily transmitted to humans through the consumption of raw dairy products and/or contact with infected animals
In addition to being a threat to public health, brucellosis can have a significant economic impact not only for animal owners by decreasing animal productivity but also for a country or region by affecting the entire production chain of meat, milk and derivatives. Although studies involving the epidemiological chain of bovine brucellosis are vast, they have not been performed with buffaloes, especially in the tropics, where countries invest little effort in the control and eradication of the disease.

Bovine brucellosis has been well studied, especially in the Americas, where the largest beef exporters in the world are located. Riveira et al. described how brucellosis was eradicated from much of Chile. However, in other countries, the prevalences vary widely, from 3-4% in Argentina, Paraguay and Central America to 5-7% in Mexico, with high rates of 10.5% to 11.4% in Venezuela and Brazil. Although epidemiological studies and health programs are common in cattle, this information is still rare in buffaloes.

Buffalo production in extensive systems, the difficulties of success in disease control programs in countries with large herds and the misconception that buffaloes are resistant to diseases that affect cattle are factors that hinder the control of brucellosis in buffaloes. Furthermore, recent studies have shown that brucellosis may have a different epidemiological profile depending on the host species.

In recent decades, bovine brucellosis has been the target of a major control and eradication program in Brazil because, in addition to production losses, this disease is one of the major health barriers to cattle export. These programs typically do not distinguish between cattle and buffaloes, adopting the same strategy for control and eradication. However, some peculiar characteristics of buffalo require specific studies for this species, especially in relation to the epidemiology, diagnosis, control and prophylaxis. Thus, the
objective of the present study was to evaluate the seroprevalence of *B. abortus* in buffalo in the states of Maranhão and Pará and in Marajó Island, Brazil.

**MATERIALS AND METHODS**

A cross-sectional prevalence study was performed, examining pregnant and non-pregnant female buffaloes with a mean age of three years from different regions of the states of Maranhão and Pará and Marajo Island, Brazil between 2010 and 2011. The animals selected for the study are part of a population of 578,500 buffaloes, corresponding to 45% of the Brazilian buffalo herd [1]. The animals from Maranhão and Pará were maintained in predominant vegetation of the Amazon rainforest (tropical forest). In the states of Pará and Maranhão, the buffaloes are predominantly raised with beef cattle. Buffaloes are vaccinated against brucellosis and foot and mouth disease, and they are periodically treated with endectocides. These animals are often maintained in production systems that seek to profit from the commercialization of meat, milk and live animals, and they are often kept in pastures of *Brachiaria brizanta* grass. In contrast, large areas of marsh and grasslands along the floodplains of rivers are found in Marajó Island. In this area, the buffaloes are raised in the wetlands; although they are vaccinated against the same agents as the animals raised in the mainland, endo- and ectoparasiticides are rarely used. These animals are raised in extensive natural pasture systems.

The minimum sample size was determined using a formula developed by the Pan American Zoonoses Center (1997) [16], as follows: 

\[ N = \frac{p(100-p)Z^2}{d^2p/100} \]

where 

- \( N \) = number of samples, 
- \( p \) = expected prevalence, 
- \( Z \) = confidence level and 
- \( d \) = margin of error.

The expected prevalence for *B. abortus* in buffalo was estimated to be 7%. The confidence
interval was 95%, and the margin of error was 5%. Thus, the minimum sample size was 4,917 animals, and 5,163 animals were used in this study. The sample consisted of 3,371 animals from Marajó Island, 1,171 animals from Pará and 221 animals from Maranhão. The animals were selected from 7 municipalities on Marajó Island, from 11 municipalities in Pará (Mainland) and from 2 municipalities in Maranhão. The animals were selected according to their size and age and the ability to access the herds.

The Rose Bengal test (RBT) was performed according to the technical manual of the National Program for Control and Eradication of Brucellosis and Tuberculosis [17]. The method consists of placing 0.03 mL of serum from the evaluated animal in contact with 0.03 mL of antigen in a gridded glass plate, mixing and then maintaining the plate in a slow and steady rotary motion until the reading. The reading is taken after four minutes of reaction, and the observation is performed with the aid of a light box. Upon agglutination, the result was determined to be positive.

The Complement Fixation Reaction was employed with incubations at 37ºC for both reaction phases. Antigen produced with *B. abortus* was used for the tube serum agglutination test. A hemolytic system was used that consisted of sheep erythrocytes sensitized with titrated hemolysin (rabbit antibody against sheep red blood cells). Tests were performed in the laboratory of the Rural Workers’ Union of Castanhal, and the antigen used was produced by the laboratory of the Institute of Technology of Paraná (Instituto de Tecnologia do Paraná - TECPAR).

The prevalence of animals that were positive for brucellosis in the RBT examination for each of the populations studied (Maranhão, Pará and Marajo Island) were evaluated using the chi-squared or Fisher's exact tests, with a confidence level of 95%. The statistical analyses were performed using the R Foundation statistical software, version 2.12.2 (2011).
RESULTS

The prevalences of seropositive animals for *B. abortus* in the RBT test were 7.37% in Marajó Island, 8.45% in Pará and 29.86% in Maranhão (Table 1).

The prevalence of *B. abortus* in buffaloes in the state of Maranhão was 4.05, which was 3.53 times higher than the prevalences observed in Pará and Marajó Island. No significant difference between the prevalences of brucellosis in Pará and Marajó Island was observed.

The municipalities with the highest prevalences for *B. abortus* in the RBT test were Santa Cruz do Arari (12.50%), Abaetetuba (20%), Ipixuna (30.25%) and Santa Inês (34.76%) (Table 2). Three municipalities in the state of Pará showed prevalences for brucellosis equal to zero.

The prevalences of buffaloes positive for brucellosis according to the confirmatory Complement Fixation test in Marajó Island, Pará and Maranhão were 0.75%, 3.88% and 0.0%, respectively (Table 3).

In the confirmatory Complement Fixation test, only 7 animals were confirmed positive in Marajó Island, and 22 were confirmed in the state of Pará. In the state of Maranhão, none of the 29.86% of animals that were positive according to the RBT test tested positive in the confirmatory Complement Fixation test.

DISCUSSION

Although buffaloes can act as an important reservoir of brucellosis for the bovine species, information on the regional prevalence and distribution of the disease in buffaloes is scarce in Brazil [13]. The last diagnosis of buffalo brucellosis in the region studied was
conducted by Láu and Singh [18], and the estimated percentages of positive animals were 5.7% in Belém, the capital of Pará, and 12.2% on Marajó Island. In the state of Maranhão, there are no studies that estimate the prevalence of brucellosis in buffaloes. Recently, Silva et al. [13] showed a prevalence of 4.8% (188/3917) for *B. abortus* in buffaloes in northern Brazil. In other states, the highest prevalences of brucellosis in buffaloes were found in São Paulo (40.9%), Goiás (20.6%) and Minas Gerais (11.0%) [19,20,21].

The lack of health control programs in Brazilian water buffalo production, the extensive management and environmental conditions of the northern region of Brazil and the misguided beliefs that buffaloes are resistant to diseases that commonly occur in cattle has made the control of brucellosis in buffaloes difficult [22]. The differences between buffaloes and cattle should be taken into consideration for the effectiveness of brucellosis control programs for water buffaloes under the abovementioned conditions [15]. Thus, new studies should be performed to understand the occurrence of this disease in areas where buffaloes and cattle are managed under the same conditions.

Bovine brucellosis has been endemic in South America [2], making the study and control of this disease in buffaloes increasingly important. With increasingly stringent health plans for the export of beef, buffaloes may become an obstacle to the success of brucellosis control and eradication programs in Brazil. Official estimates reported losses of approximately US$ 600 million/year in Latin America due to bovine brucellosis [2]. Even with the high cost of brucellosis eradication programs, a savings of US$ 7 for every US$ 1 spent on eradication was estimated. The national program of brucellosis eradication in the USA cost US$ 3.5 billion dollars between 1934 and 1997. In the USA, losses due only to reduced milk production and increased occurrence of abortions in 1952 were US$ 400 million [23].
Because buffalo production is an important economic activity in Brazil, especially in northern Brazil, the monitoring and control of brucellosis in buffaloes is essential to the success of this activity [24]. Thus, there is no doubt that serodiagnostic studies are essential and contribute to formulating an efficient control of this disease in Brazil.

CONCLUSIONS

*Brucella abortus* was found in buffaloes from the three ecosystems evaluated, thus making one of the largest water buffalo herds in the West prone to infection. The relevance of the buffalo as a source of meat, milk, leather, animal traction and trade currency for the human population in the region studied, combined with the high prevalence of the disease, makes the implementation of an effective brucellosis control program urgent to avoid the socioeconomic problems that an outbreak may cause. Moreover, these results draw attention to the danger that this disease poses to public health and for the development of buffalo production because it is common to consume unpasteurized milk in this region.

Acknowledgements

We are grateful to the office of the Dean for Research and Graduate Education of the Federal University of Pará (PROPESP/UFPA) for typing the manuscript. This study was supported by Pará State Research Foundation (FADESP). We also thank the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES) and National Council for Scientific and Technological Development (CNPq) for financial support.
Conflict of interest statement:

None of the authors has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “Detection of brucellosis in water buffaloes in northern and northeastern Brazil”.

REFERENCES


Table captions

**Table 1.** Prevalence of buffaloes positive for brucellosis according to the RBT test for the states of Maranhão and Pará and for Marajó Island, which are located in northern and northeastern Brazil, 2010-2011.

**Table 2.** Prevalence of buffaloes positive for brucellosis according to the RBT test for the municipalities evaluated in the states of Maranhão and Pará and for Marajó Island, which are located in northern and northeastern Brazil, 2010-2011.

**Table 3.** Prevalence of buffaloes positive for brucellosis according to the Complement Fixation test for the states of Maranhão and Pará and for Marajó Island, which are located in northern and northeastern Brazil, 2010-2011.
Table 1

<table>
<thead>
<tr>
<th>Locations</th>
<th>N</th>
<th>P (%)</th>
<th>PR</th>
<th>(\chi^2)</th>
<th>P-value</th>
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N = number of animals evaluated; P (%) = prevalence of serum-positive animals to \(B. abortus\); \(\chi^2\) = chi-squared; PR = prevalence ratio; ^A Reference value
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<thead>
<tr>
<th>Locations</th>
<th>N</th>
<th>P (%)</th>
<th>PR</th>
<th>$\chi^2$</th>
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N = number of animals evaluated; P (%) = prevalence of serum-positive animals to *B. abortus*; $\chi^2$ = chi-squared; PR = prevalence ratio; $^A$ Reference value.
### Table 3

<table>
<thead>
<tr>
<th>Locations</th>
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<th>PR</th>
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N = number of animals evaluated; P (%) = prevalence of serum-positive animals to B. abortus; $\chi^2$ = chi-squared; PR = prevalence ratio; $^A$ Reference value