

Detection of brucellosis in water buffaloes for exportation in northern and northeastern of Brazil*

José Diomedes Barbosa¹, Henrique dos Anjos Bomjardim¹, Danillo Henrique da Silva Lima¹, Alessandra dos Santos Belo Reis¹, Francisberto Batista Barbosa¹, Tatiane Teles Albernaz¹, Carlos Magno Chaves Oliveira¹, Adivaldo Henrique da Fonseca²⁺, Rafael Romero Nicolino³ and Jenevaldo Barbosa da Silva³

ABSTRACT. Barbosa J.D., Bomjardim H.dosA., Lima D.H.daS., Reis A.dosS.B., Barboza F.B., Albernaz T.T., Oliveira C.M.C., Fonseca A.H., Nicolino R.R. & da Silva J.B. **Detection of brucellosis in water buffaloes for exportation in northern and northeastern of Brazil.** [Detecção de brucelose em búfalos d'água para exportação no norte e nordeste do Brasil.] *Revista Brasileira de Medicina Veterinária*, 38(supl. 3):131-135, 2016. Laboratório de Doenças Parasitárias, Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural de Rio de Janeiro, BR 465, Km 7 Seropédica, RJ 23890-000, Brazil. E-mail: adivaldofonseca@yahoo.com

The prevalence of brucellosis in buffaloes was evaluated by the Buffered Acidified Plate Antigen (BAPA) in 5.163 water buffaloes from Maranhão state, Pará state and Marajó Island, Brazil. The detection of buffaloes positive for brucellosis by BAPA 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%); Ipixuna, in Pará state (30.25%); and Santa Inês, in Maranhão state (34.76%). After the confirmatory test (Complement Fixation 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 BAPA test in Maranhão reacted positively in the CF. 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.

KEY WORDS. BAPA, Buffaloes, Brazil, *Brucella abortus*.

RESUMO. A prevalência de brucelose em búfalos foi avaliada por Antígeno Acidificado Tampoadado (AAT) em 5.163 búfalos d'água dos estados do Maranhão e Pará e da Ilha do Marajó, Brasil. A detecção de búfalos positivos para brucelose por AAT foi de 7.37% na Ilha do Marajó, 8.45% no Pará e 29.86% no Maranhão. Os locais com as maiores prevalências foram Santa Cruz do Arari, na Ilha do Marajó (12.50%); Ipixuna, no Pará (30.25%); e

Santa Inês, no Maranhão (34.76%). Depois do teste confirmatório (Fixação do Complemento), apenas 7 animais permaneceram positivos na Ilha do Marajó e 22 no Pará. Nenhum dos 66 animais que reagiram positivos no teste AAT no Maranhão também reagiram positivos no FC. A alta prevalência da *B. abortus* observada nos animais, especialmente no Maranhão, é preocupante para o sistema de saúde e para o controle e erradicação da brucelose bovina.

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¹ Médico-veterinário, Instituto de Medicina Veterinária, Universidade Federal do Pará, BR 316 Km 61, Bairro Saudade, Castanhal, PA 68740-9701. E-mail: diomedes@ufpa.br; henriqueanjos18@hotmail.com; dh1sl@hotmail.com; alessandra_belo22@hotmail.com; francis_berto@hotmail.com; tatyalbernaz@ufpa.br; cmagno@ufpa.br

² Laboratório de Doenças Parasitárias, Departamento de Epidemiologia e Saúde Pública, Universidade Federal Rural de Rio de Janeiro (UFRRJ), Br 465, Km 7, 23890-000, Seropédica, RJ, Brazil. E-mail: adivaldofonseca@yahoo.com

³ Médico-veterinário, Instituto de Ciências Agrárias, Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Avenida Vereador João Narciso 1380, 38610-000, Unai, MG. E-mail: jenevaldo@hotmail.com; rafael.nicolino@gmail.com

PALAVRAS-CHAVE. AAT, búfalos, Brasil, *Brucella abortus*.

INTRODUCTION

Brazil has approximately 1.3 million buffaloes, thus constituting the largest buffalo herd in the West hemisphere (IBGE 2006). 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% (IBGE 2006).

Bovine brucellosis is a bacterial disease caused by *Brucella abortus*. The disease is one of the major zoonotic diseases in the world (Seleem et al. 2010). This disease can be easily transmitted to humans through the consumption of raw dairy products and/or contact with infected animals (Brasil 2006). 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 (Acha et al. 2003; Seleem et al. 2010). 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 (Megid et al. 2000).

Bovine brucellosis has been well studied, especially in the Americas, where the largest beef exporters in the world are located. (Rivera et al. 2002) described how brucellosis was eradicated from much of Chile. However, in other countries, the prevalence vary widely, from 3-4% in Argentina (Samartino 2002), Paraguay (Baumgarten 2002) and Central America (Moreno 2002) to 5-7% in Mexico (Luana-Martínez & Mejía-Terán 2002), with high rates of 10.5% to 11.4% in Venezuela (Francisco & Vargas 2002) and Brazil (Borba et al. 2013). Although epidemiological studies and health programs are common in cattle, this information is still rare in buffaloes (Silva et al. 2014).

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 (Nardi Jr et al. 2012). Furthermore, recent studies have shown that brucellosis may

have a different epidemiological profile depending on the host species (Fosgate et al. 2002).

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, Pará and Marajó 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 (IBGE 2006). 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 frequently kept in pastures of *Brachiaria brizantha* 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 study used a convenience sample, consisting of 5,163 animals selected for export. 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 provinces on Marajó Island, from 11 provinces in Pará (Mainland) and from 2 provinces in Maranhão. The animals were selected according to the criteria determined by the importing country, based on preliminary on zootechnical standards, and later conducting diagnostic tests for tuberculosis and brucellosis. In the origin farms, the selected animals based on zootechnical standards were submitted to the screening test - Buffered Acidified Plate Antigen - BAPA, and the positive animals were discarded. Negative animals were transferred to a quarantine and subjected to the Com-

plement Fixation tests or Fluorescent Polarization assay, as confirmatory for export, once again, the positive animals were discarded for export. All diagnostic protocol was defined by the importing country.

BAPA was performed according to the technical manual of the National Program for Control and Eradication of Brucellosis and Tuberculosis of Brazil (Brasil, 2006). 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 37°C 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 BAPA examination for each of the populations studied (Maranhão, Pará and Marajó 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 prevalence of seropositive animals for *B. abortus* in the BAPA test were 7.37% in Marajó Island, 8.45% in Pará and 29.86% in Maranhão (Table 1 and Figure 1).

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

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

Table 1. Prevalence of buffaloes positive for brucellosis according to the Buffered Acidified Plate Antigen (BAPA) test for the states of Maranhão, Pará and for Marajó Island, which are located in northern and northeastern Brazil, 2010-2011.

Locations	N	P (%)	PR	χ^2	P-value
MarajóA	3771	7.37	-	-	-
Pará	1171	8.45	0.87	1.51	0.243
Maranhão	221	29.86	0.25	134.39	0.000

N = number of animals evaluated; P (%) = prevalence of serum-positive animals to *B. abortus*; χ^2 = chi-squared; PR = prevalence ratio; A Reference value

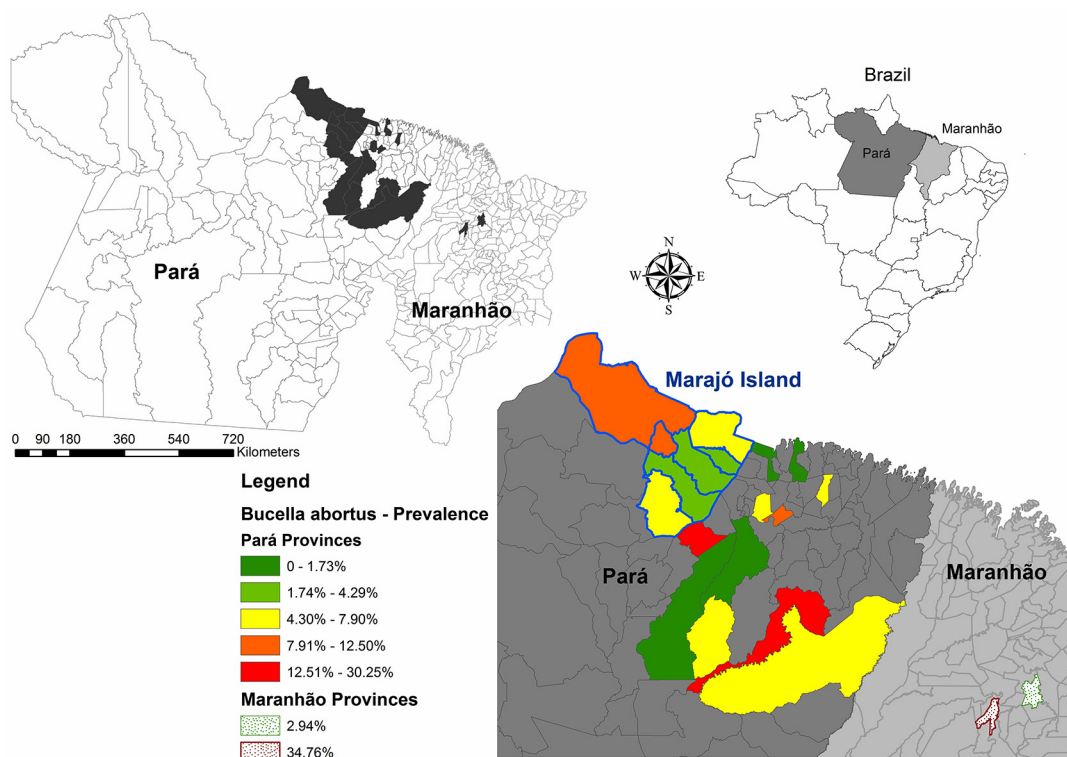


Figure 1. Prevalence of brucellosis on buffaloes - Buffered Plate Antigen - 2010/2011 Maranhão, Pará and Marajó island, Brazil.

Table 2. Prevalence of buffaloes positive for brucellosis according to the Buffered Acidified Plate Antigen (BAPA) test for the provinces evaluated in the states of Maranhão, Pará and for Marajó Island, which are located in northern and northeastern Brazil, 2010-2011.

Locations	N	P (%)	PR	χ^2	P-value
Marajó Island					
Cachoeira do ArariA	306	4.08	-	-	-
Chaves	255	9.36	0.44	5.57	0.0307
Muaná	245	5.84	0.70	0.73	0.5332
Ponta de Pedras	183	2.35	1.74	0.55	0.6720
Salvaterra	234	4.29	0.95	0.01	0.8806
Santa C. Arari	364	12.50	0.33	15.89	0.0001
Soure	2184	7.24	0.56	4.42	0.0471
Pará					
Abaetetuba	36	20.00	-	-	-
Acará	47	0	-	6.75	0.0094
Inhangapi	33	9.09	2.20	1.01	0.0063
Ipixuna	83	30.25	0.66	0.87	0.5028
Marapanim	37	0	-	1.64	0.5068
Mojú	170	1.73	11.56	17.11	0.0005
Nova Timboteua	62	6.06	3.30	3.53	0.1497
Paragominas	443	7.90	2.53	3.66	0.1332
Santa Isabel	36	5.88	3.40	1.56	0.4417
São C. Odívalas	42	0	-	4.86	0.0932
Tailândia	182	5.70	3.50	5.66	0.0548
Maranhão					
Vitória do Miarim ^A	99	2.94	-	-	-
Santa Inês	122	34.76	0.08	13.90	0.000

N = number of animals evaluated; P (%) = prevalence of serum-positive animals to *B. abortus*; χ^2 = chi-squared; PR = prevalence ratio; ^A Reference value

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

Locations	N	P (%)	PR	χ^2	P-value
Marajó Island ^A	935	0.75	-	-	-
Pará	567	3.88	0.19	16.62	0.001
Maranhão	122	0	-	0.91	0.714

N = number of animals evaluated; P (%) = prevalence of serum-positive animals to *B. abortus*; χ^2 = chi-squared; PR = prevalence ratio; ^A Reference value

The prevalence 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 BAPA 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 (Silva et al. 2014). The last diagnosis of buffalo brucellosis in the region studied was conducted by (Lau & Singh 1986), 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. 2014) 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%) (Costa et al. 1973, Santa Rosa 1961).

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 (Guarino et al. 2001). 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 (Fosgate et al. 2002). 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 (Seleem et al. 2010), 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 (Seleem et al. 2010). 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 (Vishvanath & Kole 2008).

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 (Paulin & Ferreira Neto 2008). 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. 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.

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