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ORIGINAL ARTICLE

Serological survey of *Mycobacterium bovis, Brucella abortus* and *Borrelia burgdorferi* in water buffaloes in the northern region of Brazil

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ABSTRACT: The largest buffalo herds are in the northern region of Brazil, so few studies have been conducted to assess the prevalence of selected parasitic diseases in buffalo herds. The present study was therefore conducted to research the epidemiology of *Borrelia burgdorferi*, *Mycobacterium bovis* and *Brucella abortus* in water buffaloes in the north region of Brazil. A total of 4796 buffalo blood samples were randomly collected from five provinces and simultaneously analyzed by ELISA, tuberculin test, CFT and 2ME. The occurrence of *B. burgdorferi* in buffalo was 75% by ELISA. The tuberculin testing prevalence of *M. bovis* was 4.6%. The overall prevalence of *B. abortus* was 4.8% and 4.6% by CFT and 2ME, respectively. Thus, it is concluded that all agents studied were circulating in buffaloes. Special emphasis should be given to brucellosis and tuberculosis agents which are important to public health. Another important finding was the high titers of antibodies found against borreliosis agents, but a possible agent and vector have not been identified yet in Brazil.

Key words: borreliosis, brucellosis, prevalence, serology, tuberculosis.

Encuesta serológica de *Mycobacterium bovis, Brucella abortus* y *Borrelia burgdorferi* en búfalos de agua en la región norte de Brasil

RESUMEN: Aunque los mayores rebaños de búfalos están en la región norte de Brasil, se han realizado pocos estudios para evaluar la prevalencia de determinadas enfermedades parasitarias. El presente estudio se realizó para investigar las características epidemiológicas de *Mycobacterium bovis*, *Brucella abortus* y *Borrelia burgdorferi* en búfalos de agua en la región norte de Brasil. Un total de 4796 muestras de sangre de búfalo fueron seleccionadas al azar en cinco provincias y se analizaron simultáneamente a través de ELISA, CFT, 2ME y prueba de tuberculina. La prevalencia global de *B. abortus* fue de 4,8% y 4,6% en CFT y 2ME, respectivamente. La prevalencia de pruebas de tuberculina de *M. bovis* fue del 4,6%. La ocurrencia de *B. burgdorferi* en búfalos fue del 75% por ELISA. Por lo tanto, se llega a la conclusión de que todos los agentes estudiados están circulando en los búfalos de esta región. Se le debe dar especial énfasis a los agentes de la brucelosis y la tuberculosis, que son de importancia para la salud pública. Otro hallazgo importante fue los altos títulos de anticuerpos que se encuentran contra la borreliosis, pero aún no se ha identificado un posible vector en Brasil.

Palabras clave: borreliosis, brucelosis, prevalencia, serología, tuberculosis.

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INTRODUCTION

Brazil has the biggest western buffalo herds, where approximately the 65% is located in the northern region of this country (1). Nowadays, the buffalo has been highlighted in the national scenery, showing just an alternative to the occupation of lands unsuitable for cattle, but becoming an economically important option. Consequently, a concern about a sanitary management has increased considerably, because the clinical, pathological and epidemiological studies are still poorly studied in Latin America.

Buffaloes, when compared to other domestic livestock, are generally resistant animals (2). This is particularly impressive because most of them, especially water buffaloes, live in hot and humid regions which have several infectious agents (2). Although the reason is not clear, the effect on buffaloes is often less deleterious than that on cattle.

According to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organisation for Animal Health (OIE), brucellosis and tuberculosis are the most important and widespread zoonoses in the world. Lyme disease, the most common tick-borne disease in North America and Europe, is caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato species complex (3).

Although many studies have been conducted worldwide on the prevalence of these important pathogens in humans and animals, few studies have been conducted on water buffaloes in Latin America. The present work is aimed at assessing the prevalence of *Brucella abortus*, *Mycobacterium bovis* and *B. burgdorferi* among water buffaloes in the northern region of Brazil.

MATERIALS AND METHODS

Serum samples

Field samples of blood from water buffalo (n = 4796) were collected from different farms in seven provinces in Marajó island (Soure, Salvaterra, Muaná, Chaves, Ponta de Pedras, Cachoeira do Arari and Santa Cruz do Arari) in the northern region of Brazil in 2011. Whole blood samples were collected from caudal or jugular vein of individual water buffaloes. For serum samples, blood samples without EDTA were incubated at room temperature and then centrifuged at 3000 rpm for 15 min; sera were collected and stored at -20ºC until use.

The number of samples to assess the prevalence of *B. abortus, M. bovis* and *B. burgdorferi* in the northern region of Brazil was determined using the formula recommended by the Pan American Zoonosis Center (CEPANZO) (4) for the study of chronic diseases: N=p.(100-p)Z2/(d.p/100)2, where n means number of samples; p, expected prevalence; Z, confidence level, and d, error margin. A total of 4796 samples was determined based on estimated prevalence of 7% of positive samples to *B. abortus*, ascertained in a pilot study with 100 samples, where a confidence level of 95.0% and an error margin of 5.0% were established.

Enzyme-Linked Immunosorbent Assays (ELISA) for *Borrelia burgdorferi*

A G39/40 of *B. burgdorferi* isolate from USA, kindly supplied by Dr. Natalino Hajime Yoshinari, was used for ELISA crude antigen production (5, 6).

Briefly, 100 µl of each antigen diluted in 0.05 M carbonate/bicarbonate buffer, pH 9.6, were added to each well using a micro-ELISA plate (Immulon®; Dynatech Laboratories Inc.); and protein concentration was adjusted to 10 µg/mL. Plates were sealed and incubated overnight at 4°C; then blocked for l h at 37°C in a humid chamber with 3% skim milk in carbonate/bicarbonate buffer. After five washes with PBS-Tween buffer (phosphatebuffered saline, pH 7.2, and 0.05% Tween 20), 100 µl of diluted bovine sera (1:400) in PBS-Tween plus 5% normal rabbit serum were added in duplicate to the ELISA plate. Plates were incubated at 37°C in a humid chamber for 90 min and then washed five times with PBS-Tween. A 100 µl aliquot of a 1:25000 dilution in PBS-Tween of alkaline phosphatase conjugated anti-bovine IgG (Sigma, St. Louis, Missouri, USA) was added to each well and plates were incubated at 37°C under the same conditions for 90 min. Plates were then washed five times with PBS-Tween. The appropriate substrate 4-Nitrophenyl phosphate disodium salt hexahydrate (Sigma, St. Louis, Missouri, USA) was added and plates were sealed and incubated for 40 min at room temperature. Then they were read at 405 nm wavelength on a micro- ELISA reader (B.T.-100; Embrabio, São Paulo, Brazil). The cut-off values were calculated based on 10 non-infected water buffaloes sera by the receiver operating characteristic (ROC) analysis with MedCalc statistical software (version 11.4; http://www.medcalc.be).

Tuberculin testing for *Mycobacterium bovis*

Tuberculosis testing was performed using purified protein derivative (PPD) (Laboratório Tecpar, starting number 07/11 and manufacture 07/11, Brazil). Volumes of 0.1 ml of 30,000 IU/ml bovine PPD and 25,000 IU/ml avian PPD were used. An animal was classified as positive if the swelling of the skin fold at the bovine site was higher than that found in the avian site by 4 mm.

Herds were classified as positive tuberculosis if they had at least one positive bovine reactor animal.

Complement fixation test (CFT) and 2-mercaptoethanol (2ME) for *Brucella abortus*

The mercaptoethanol agglutination test (2ME) was performed following USDA method by adding 80, 40, 20 and 10 mL of serum to four tubes. One mL of 0.1 M mercaptoethanol in 0.85 g% NaCl and 1 mL of double strength (1:100) standard tube agglutination antigen in 0.85 g% NaCl were added. Tubes were shaken and incubated, and reactions were read as for the standard tube agglutination.

A microtiter cold complement fixation test (CFT) and an automated complement fixation test were performed as previously described (7). The buffered plate antigen test was performed with antigen provided by the Institute of Technology of Paraná, Brazil. There was an 11% suspension of *B. abortus* stained with crystal violet and brilliant green and buffered to pH 3.63. The test was performed as the standard plate agglutination tests, mixing 80 µl of serum and 30 µl of antigen. The incubation time was eight minutes, with the plate being rotated four times after four minutes of incubation. Reactions were read as ++ for complete agglutination and + for partial agglutination. A negative reaction was a homogenous serum-antigen mixture with no evidence of agglutination.

Statistical analysis

Kappa coefficient was calculated to evaluate the agreement between CFT–2ME. Chi-square test was used to evaluate significant differences ($p<0.05$) of infection rate (*B. abortus, M. bovis* and *B. burgdorferi*) in animals from different breeds, reproductive status and locations. The operational procedures were carried out using the R statistical software (R Foundation for Statistical Computing, version 2.12.2, 2011).

RESULTS

IgG antibodies to *B. burgdorferi* were detected in 75% (3597/4796) of the buffaloes sampled assessed by ELISA test (Figura 1). One hundred-eighty out of 4796 (3.75%) of the buffaloes sampled showed positive results in tuberculin test (Figure 1). In the complement fixation test, screening test, 3.81% (183/4796) of sampled animals showed positive results for *B. abortus*. In the confirmatory 2-ME test, the 3.67% (176/4796) of buffaloes showed positive results (Figure 1). Therefore, 38 buffaloes were identified as false positive by -ME test (Kappa index 0.9). The number of samples, positive to *B. abortus*, *M. bovis* and *B. burgdorferi,* was 1.67% (80/4796). The number, positive to *B. abortus* and *M. bovis,* was 1.40% (67/4796), positive to *B. abortus* and *B. burgdorferi* was 2.84% (136/4796) and positive to *M. bovis* and *B. burgdorferi* was 3.25% (156/4796).

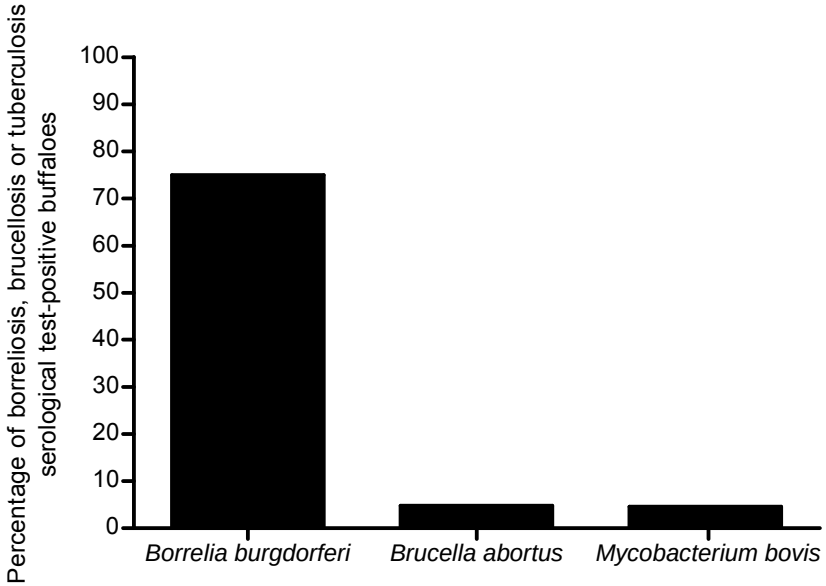

FIGURE 1. Serological detection of *Brucella abortus, Mycobacterium bovis* and *Borrelia burgdorferi* in water buffaloes from the northern region of Brazil./ *Detección serológica de* ***Brucella abortus, Mycobacterium bovis y Borrelia burgdorferi*** *en búfalos de agua de la region norte de Brasil.*

DISCUSSION

In Brazil, serological surveys on domestic animals have already detected antibodies to *B. burgdorferi* in horses (8) and cattle (9) in Pará state, and dogs (10) and buffalos (6) in Rio de Janeiro state. The frequency of seropositive buffaloes in the present work was markedly higher than that found among cattle (54.9%) (9) and horses in (26.7%) (8). These observed differences in seropositivity to *B. burgdorferi* may be due to a higher susceptibility found in buffaloes to this agent associated to intrinsic immunological factors (6).

Previous studies in the northern region of Pará state reported a prevalence of 5.7% and 12.2% for *B. abortus* among buffaloes in the continent and Marajo Island, respectively (11). The highest prevalence of *B. abortus* previously found could be explained by the fact that the buffaloes sampled showed a history of abortion, retention of placenta, endometritis and articular hydroma (11). Herein, animals sampled did not show clinical signs of brucellosis and history of abortions.

Retrospective studies have shown that bovine tuberculosis is prevalent in throughout Latin America and the Caribbean (12). The group with a relatively high prevalence or no information reported comprises several South American countries such as Argentina, Bolivia, Brazil, Chile, Ecuador, Peru and Guyana. The prevalence found in the present study was lower than that found among buffaloes from São Paulo (8.11%) (13) and Amazônia (20.4%) (142).

Although Brazil has the largest commercial cattle herds in the world and the largest herds of buffaloes in the western region (15), few studies have been conducted to ascertain the prevalence of tuberculosis and brucellosis in buffaloes. The real impact of *M. bovis* and *B. abortus*-infected buffaloes on cattle production needs to be determined. Although buffaloes are not yet part of the Brazilian trade balance, they are considered a major source of protein in Marajó Island and may play a role as reservoirs for *M. bovis* and *B. abortus*.

CONCLUSION

The present study provides important information about the prevalence of *M. bovis*, *B. abortus* and *B. burgdorferi* infections in water buffaloes. The real role of water buffaloes on the epidemiology of these diseases, and consequently, the impact of management and control programs targeting these animals should be determined.

CONFLICT OF INTEREST STATEMENT

None of the authors of this work has a financial or personal relationship with other people or organizations that could inappropriately influence on the content of the paper.

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