

ORIGINAL ARTICLE

Serological prevalence and risk factors of *Borrelia burgdorferi* in water buffaloes (*Bubalus bubalis*, Linnaeus, 1758) on Marajó Island, northern region of Brazil

Jenevaldo Barbosa da Silva^{1*}, Bruna de A. Baêta^{II}, Cinthia T. A. Lopes^{III}, Bruna Sampaio Martins Land Manier^{II}, Gustavo Nunes Santana de Castro^{II}, Priscilla Nunes dos Santos^{II}, Adivaldo Henrique da Fonseca^{II}, José Diomedes Barbosa^{III}

^IFaculdade de Ciências Agrárias e Veterinárias - UNESP, Rod. Carlos Tonanni, km 05, 14870-000, Jaboticabal, São Paulo, Brazil. E-mail: jenevaldo@hotmail.com. ^{II}Universidade Federal Rural do Rio de Janeiro - UFRRJ, BR465, Km 07, 23890-000, Seropédica, Rio de Janeiro, Brazil. E-mail: adivaldofonseca@yahoo.com. ^{III}Universidade Federal do Pará, Centro Agropecuário, Departamento de Ciência Animal. Rua Maximino Porpino da Silva, 1000, Centro Castanhal, PA, Brazil. 68748-080. E-mail: diomedes@ufpa.br.

ABSTRACT: Sera samples were collected from 330 water buffaloes on Marajó Island, state of Pará, Brazil, to assess the presence of antibodies against *Borrelia burgdorferi* by indirect Enzyme Linked Immunosorbent Assay. Approximately 45% of the animals had antibodies against *B. burgdorferi*. The prevalence of seropositive buffaloes, 72% (85/118), was statistically higher in the city of Soure than in the other municipalities tested. Murrah breed animals were significantly more seropositive (Prevalence Ratio (PR) = 1.84, $p = 0.000$) than those of the Mediterranean breed. Among the animals diagnosed positive for tuberculosis, 33% (4/12), were also seropositive for *B. burgdorferi*. Animals positive for tuberculosis had a significantly lower level of *B. burgdorferi* seropositivity (PR = 1.36, $p = 0.0017$) than negative animals. The study showed that there was an agent immunologically close to *B. burgdorferi* circulating in water buffalo on Marajó Island, state of Pará, Brazil. These results demonstrate a possible role of the water buffalo in the epidemiological chain of this agent in Brazil, and are a warning of the danger this disease could pose to public health.

Key words: spirochetes, *Borrelia* sp., ELISA, buffaloes, risk factors.

Prevalencia serológica y factores de riesgo de *Borrelia burgdorferi* en búfalos de agua (*Bubalus bubalis*, Linnaeus, 1758) en la isla Marajó, región norte de Brasil

Resumen: Para determinar la presencia de anticuerpos contra *Borrelia burgdorferi* por iELISA, se colectaron 330 muestras de suero de búfalos de agua en la Isla Marajó, estado de Pará, Brasil. Aproximadamente el 45% de los animales tuvieron anticuerpos contra *B. burgdorferi*. La prevalencia de búfalos seropositivos, 72% (85/118), fue estadísticamente más alta en la ciudad de Soure que en el resto de las municipalidades estudiadas. Los animales de raza Murrah fueron significativamente más positivos (ratio de prevalencia (RP) = 1.84, $p = 0.000$) que los de la raza Mediterránea. Entre los animales diagnosticados positivos para tuberculosis, 33% (4/12) también fueron seropositivos para *B. burgdorferi*. Los animales positivos para tuberculosis tuvieron un nivel significativamente menor de seropositividad para *B. burgdorferi* (PR = 1.36, $p = 0.0017$) que los animales negativos. El estudio mostró que existe un agente inmunológicamente relacionado con *B. burgdorferi* circulando en búfalos de agua en la isla Marajó, estado de Pará, Brasil. Estos resultados demuestran el posible papel del búfalo de agua en la cadena epidemiológica de este agente en Brasil y que constituye una advertencia sobre el daño que esto puede ocasionar a la salud pública.

Palabras clave: espiroquetas, *Borrelia* sp., ELISA, búfalos, factores de riesgo.

*Correspondencia: Jenevaldo Barbosa da Silva. E-mail: jenevaldo@hotmail.com.

INTRODUCTION

In 1890, the water buffalo arrived in Brazil at Marajó Island, Pará state; today they are found throughout the country and constitute the largest herd in the West (1), with one third of the national herd still remaining on the island of Marajó (2).

The genus *Borrelia* (Swellengrebel, 1907) consists of Gram negative, microaerophilic, mobile bacteria, classified in the order Spirochaetales and family Spirochaetaceae. Lyme borreliosis, caused by spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex, is an anthroponosis referred to as Baggio-Yoshinari syndrome in Brazil (3). Ticks act as vectors, becoming infected upon ingestion of blood from an infected vertebrate host, and showing transovarial and transtadial transmission (4, 5, 6). Wild animals, including deer, rodents and birds, represent important reservoirs of the spirochete (7, 8).

Serological analysis by Enzyme Linked Immunosorbent Assay (ELISA) has a wide application in epidemiological studies of Lyme borreliosis because a large number of samples can be concomitantly analyzed at low cost; however, it has low specificity and cross reactions may occur (9, 10).

In Brazil, previous studies have confirmed the presence of antibodies against *B. burgdorferi* in several animal species such as cattle (11, 12), horses (13), and dogs (14, 15). The first report of *Borrelia* sp. in water buffaloes was from the state of Pará, when Scofield *et al.* (16) observed morphometric spirochetes in a buffalo with clinical suspicion of Enzootic Bovine Leukosis. In 2012, Corrêa *et al.* (17) found 83.9% of buffaloes seropositive for *B. burgdorferi*; however, the possible risk factors for infection are still unknown. Therefore, the objective of this study was to use indirect ELISA to evaluate the presence of antibodies against *B. burgdorferi* in water buffaloes on Marajó Island, state of Pará, and their association with potential risk factors.

MATERIALS AND METHODS

The study was conducted in the municipalities of Soure, Muaná, Salvaterra, Thailand Ponta de Pedra, and Santa Cruz Arari during the year 2012. All the municipalities belong to Marajó Island, Pará, located at latitude 00° 43'00 «south and longitude 48° 31'24 «west, with an altitude of 10 meters.

Blood samples were collected by coccygeal vein puncture from 330 randomly selected buffaloes aged between two and three years. Of these animals, 156 were of Mediterranean and 174 of Murrah breed. Among

all animals studied, 123 were pregnant. Twelve buffaloes tested positive for tuberculosis, and 15 were positive for brucellosis.

The enzyme-linked immunosorbent assay was based on the description of Silva *et al.* (12). The antigen was prepared from *B. burgdorferi* sensu stricto G 39/40 of North American origin and adjusted to 5 µg/ml protein to coat plates by overnight incubation at 4°C, after which the plates were blocked for 90 minutes at 37°C. Test samples were diluted 1:400 in PBS-Tween 20 with 5% milk powder, and incubated in plates at 37°C in a humid chamber for 90 minutes. After this, 100µl of the alkaline phosphatase anti-bovine IgG (Sigma Chemical Co.), diluted to 1:30000 according to manufacturer's recommendations, was added to each well. The plates were incubated in the same conditions as before. The substrate p-nitrophenyl phosphate (PNPP, Sigma Chemical Co.) was added at 100µl/well and the plates were incubated at room temperature for 50 minutes, after which the absorbance was read at 405 nm in a micro-ELISA reader (Labsystems iEMS Reader MF).

Cut-off values were calculated based on 30 non-*Borrelia*-infected water buffalo sera by the receiver operating characteristic (ROC) analysis with MedCalc statistical software (version 11.4; <http://www.medcalc.be>) (18). Thirty serum samples obtained from foals before colostrum suckling were used as negative controls. A positive reference group consisting of 10 buffalo serum samples tested positive for *B. burgdorferi* (titres of 1280 by IFAT) were used as positive controls in the serological assays.

The chi-square test was used to determine significant differences in the percentages of samples tested, at a significance level of 5%. The association between seropositivity to *B. burgdorferi* and risk factors of locality, breed, physiological state (pregnancy) and positivity to tuberculosis and brucellosis was performed using the chi-square (+2). The analysis was performed by the statistical software R Foundation for Statistical Computing, version 2.12.2 (2011).

RESULTS

Among the 330 buffalo serum samples analyzed, 148 animals had antibodies against *B. burgdorferi*, representing a prevalence of 44.8%. The number of seropositive animals varied significantly ($p < 0.05$) depending on the location, breed and positivity for tuberculosis (Table 1).

The prevalence of seropositive buffalo found in the city of Soure (72%) was statistically higher than in the

TABLE 1. Evaluation of risk factors for the presence of antibodies against *B. burgdorferi* in water buffaloes on Marajó Island, state of Pará, Brazil./ *Evaluación de factores de riesgo por la presencia de anticuerpos contra B. burdoferi en búafalos de agua en la isla Marajó, estado de Pará, Brasil.*

Risk factors	N	P (%)	χ^2	PR	p-valor	CI 95%
locality						
(1 Soure*	118	72%	-	-	-	-
(2 Muaná	41	20%	7.0445	3.69	0.0079	0.0499 - 0.3383
(3 Salvaterra	39	31%	0.0588	2.34	0.0234	0.1794 - 0.1192
(4 Tailândia	25	40%	0.0321	1.80	0.0003	0.0996 - 0.8773
(5 Ponta de Pedra	20	20%	43.2842	3.60	4.734 ^{e-11}	0.2959 - 0.5362
(6 Santa Cruz do Arari	87	33%	34.0338	2.16	5.416 ^{e-09}	0.2899 - 0.5486
breed						
(1 Mediterrâneo*	156	31%	-	-	-	-
(2 Murrah	174	57%	22.324	0.55	2.303 ^{e-06}	0.1571 - 0.3758
brucellosis						
(1 Positive*	15	40%	-	-	-	-
(2 Negative	315	45%	0.3548	0.89	0.5514	0.1132 - 0.0551
tuberculosis						
(1 Positive*	12	33%	-	-	-	-
(2 Negative	318	45%	9.8137	0.74	0.0017	0.2248 -0.0540
pregnancy						
(1 Positive*	123	41%	-	-	-	-
(2 Negative	207	47%	0.6377	0.86	0.4245	0.1567 - 0.0607

other municipalities studied. Murrah animals were significantly more seropositive (PR = 1.84, p = 0.000) than the Mediterranean breed animals. Among animals positive for tuberculosis, 33% (4/12) were also seropositive for *B. burgdorferi*, a significantly lower level of seropositivity (PR = 1.36, p = 0.0017) than that seen in tuberculosis-negative animals (Table 1).

DISCUSSION

The number of seropositive animals observed in this study indicates the presence of a spirochete immunologically similar to *B. burgdorferi* in buffaloes in the region studied, as previously suggested by Scofield *et al.* (16) and Corrêa *et al.* (17). The frequency of seropositive buffaloes in this study was lower than the frequency observed by Corrêa *et al.* (17) in working with Murrah buffalo from the mainland and Marajó Island, in the state of Pará, Brazil. This study showed that Murrah buffaloes were more likely to have antibodies against *B. burgdorferi* than the Mediterranean breed, explaining the lower rate of positive animals seen here, when both breeds were studied, compared with Corrêa *et al.* (17). In a study on the same region, Guedes-Junior *et al.* (11) found a similar seroprevalence (54.9%) of *B. burgdorferi* in cattle.

In a study of cattle, Kocan *et al.* (19) found a limitation in the use of the European breed *Bos taurus*, it does not have antibodies against tick-borne diseases. In contrast, *Bos indicus*, of Indian origin, has a greater degree of resistance, because of the immunity it acquired, for its greater contact with agents. The same idea can be applied to the Mediterranean and Murrah breeds of buffalo, since the Mediterranean breed of European origin, as with *Bos taurus*, showed lower seropositivity, whereas Murrah animals (Indian origin) had higher levels of seropositivity than *B. burgdorferi*.

The drop in immunity caused by tuberculosis (20) impairs the immune response against other agents - this could explain the association observed between animals positive for tuberculosis and seronegative against *B. burgdorferi*.

CONCLUSIONS

This study demonstrates the existence of an immunologically similar agent to *B. burgdorferi* circulating in water buffaloes on Marajó Island, state of Pará, Brazil. The lower prevalence of antibodies against *B. burgdorferi* in buffaloes positive for tuberculosis may indicate the importance of this debilitating disease in impairing the immune response not only to borreliosis,

but possibly to other agents as well. Future studies on buffaloes should consider the importance of mixed infections by different agents.

Differential breed susceptibility is already known in cattle, but there have not been similar studies on buffaloes. Although we observed that Murrah buffaloes were more susceptible than the Mediterranean buffaloes, more detailed studies should be conducted to evaluate the immunological basis of this finding.

These results demonstrate the possible role of the buffalo in the epidemiological chain of this disease in Brazil, and serve as a warning of the danger that this disease poses to public health.

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 or bias the content of the paper.

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