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Detection of anti-*Borrelia burgdorferi* antibodies in buffaloes (*Bubalus bubalis*) in the state of Pará, Brazil

Detecção de anticorpos anti-*Borrelia burgdorferi* em búfalos (*Bubalus bubalis*) no estado do Pará, Brasil

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Abstract

This study aimed to investigate the frequency of homologous antibodies of IgG class against *Borrelia burgdorferi* in buffaloes in the state of Pará, Brazil. Blood serum samples from 491 buffaloes were analyzed by means of the indirect ELISA test, using crude antigen produced from a cultivar of the North American strain G39/40 of *B. burgdorferi*. There were 412 positive samples (83.91%), and there was no statistically significant difference in the proportions of positive animals between the 81.69% (232/284) originating from Marajó Island and the 86.96% (180/207) from the continental area of the state of Pará. In all the municipalities studied, the frequency of positive findings of antibodies against *B. burgdorferi* among the animals ranged from 63.6% to 92.9%. The high numbers of seropositive animals can be explained by the frequent presence of the tick *Rhipicephalus (Boophilus) microplus*, and by the possible existence of spirochetes of the genus *Borrelia* infecting buffaloes in the region studied, although specific studies are needed to confirm this relationship. These factors suggest that a cross-reaction exists between the North American strain G39/40 of *B. burgdorferi*, which is used as an antigenic substrate, and the species of *Borrelia* spp. that possibly infects buffaloes in the state of Pará.

Keywords: Buffaloes, *Borrelia* spp., epidemiology, serology.

Resumo

Este estudo teve como objetivo investigar a frequência de anticorpos homólogos da classe IgG contra *Borrelia burgdorferi* em búfalos do estado do Pará. Amostras de soro de 491 búfalos foram analisadas por meio do teste ELISA indireto, utilizando antígeno bruto produzido a partir do cultivo da cepa norte americana G39/40 de *B. burgdorferi*. Foram encontrados 412 soros positivos (83,91%), não havendo diferença estatística significativa entre os 81,69% (232/284) animais positivos provenientes da Ilha de Marajó e os 86,96% (180/207) da base continental do estado do Pará. Em todos os municípios estudados os animais apresentaram frequência de anticorpos contra *B. burgdorferi*, com positividade variando de 63,6% a 92,9%. O alto número de soropositivos pode ser explicado pela frequente presença do carrapato *Rhipicephalus (Boophilus) microplus* e pela possível existência de espiroquetas do gênero *Borrelia* infectando búfalo na região estudada, embora novos estudos sejam necessários para a confirmação desta relação. Estes fatos sugerem reação cruzada entre a cepa americana G39/40 de *B. burgdorferi* utilizada como substrato antigênico e a espécie de *Borrelia* spp. que possivelmente infecta bubalinos no estado do Pará.

Palavras-chave: Búfalos, *Borrelia* spp., epidemiologia, sorologia.

Introduction

Borreliosis is a systemic anthropozoonosis, an infectious spirochetal disease of the group *Borrelia burgdorferi sensu lato* that is found in North America and Europe (RUDENKO et al., 2009). Similarly to what is observed in humans, these microorganisms can infect species of domestic animals and wildlife, and they are transmitted primarily by ticks (LITTLE et al., 2010). Various tick species have been described as parasitizing buffaloes (NITHIKATHKUL et al., 2002), which makes them possible hosts for the etiological agents transmitted by these arthropods.

Buffalo production in Brazil is an activity that has been attracting the attention of the scientific community with regard to aspects

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of buffalo health. Buffaloes have anatomical and physiological characteristics that confer on them great rusticity, which facilitates development of the subclinical state of many infections (LÁU, 1999). The theory that buffaloes are highly resistant to diseases has led to their health being treated with little attention, without taking into consideration the possibility that buffaloes may participate in maintaining the epidemiological cycle of etiological agents of diseases of importance to livestock and public health.

The first observation of *Borrelia* spp. in buffaloes was made in a native buffalo in the municipality of Castanhal, state of Pará. Guedes Junior et al. (2008) found 54.9% (135/246) cattle positives to homologues antibodies to *B. burgdorferi*. (GALO et al., 2009), found 26,7% (80/300) horses positives to homologues antibodies to *B. burgdorferi*, both in the same region. On the basis of this finding, the current study aimed to evaluate the frequency of homologous anti-*B. burgdorferi* antibodies, using the indirect enzymatic immunoabsorption (ELISA) test, on blood serum samples from buffaloes in the state of Pará, Brazil.

Materials and Methods

For this study, blood samples were collected from 491 young and adult buffaloes of the Murrah breed that were apparently healthy. Animal recruitment was carried out by non-probabilistic sampling. Among these animals, 284 came from the municipality of Cachoeira do Arari, Marajó Island. A further 207 animals came from four municipalities situated in the continental part of Pará: 66 animals from Castanhal, 22 from Santa Isabel, 63 from Santarém Novo and 56 from Nova Timboteua. The animals' blood was obtained in an aseptic form by means of jugular venal puncture and was decanted into sterile tubes without anticoagulant. The serum was sampled into microtubes and stored at –20 °C until it was serologically analyzed.

The antigen used for serological tests came from a cultivar of the G39/40 strain of *B. burgdorferi stricto sensu*, of North American origin. Spirochetes were put to grow in Barbour-Stoenner-Kelly (BSK-H) medium and, at maximal bacterial increase, antigens were produced as described by Ishikawa et al. (1997). The *B. burgdorferi* G39/40 strain was kindly provided by Professor Dr. Natalino Hajime Yoshinari, of the Medical Investigation Laboratory for Rheumatology, Hospital das Clínicas, School of Medicine of the University of São Paulo (LIM-17/HCFMUSP).

Prior to the serological analyses, validation of the indirect ELISA test standardized by Ishikawa et al. (1997) was performed. In a high adsorption ELISA plate (Costar 3590, Corning®), tests were carried out with various antigen concentrations (10 µg/mL, 15 µg/mL and 20 µg/mL); two serum dilutions (1:400 and 1:800), of which two were positive and two were negative in duplicate; and two dilutions of rabbit anti-bovine IgG conjugated to alkaline phosphatase (Sigma Chemical®) (1:5000 and 1:10000). The results obtained by means of spectrophotometry were expressed as optical density values (OD). The best combination for producing the greatest difference between the positive and the negative serum samples was chosen. The ELISA assay on buffalo serum samples was done using the technique described by Ishikawa et al. (1997), with modifications.

The cutoff for each ELISA plate was calculated according to the mathematical formula of Frey et al. (1998), which is based on a factor t (Student t distribution) determined by the number of negative controls and the desired confidence level. This study used serum samples from 12 healthy buffaloes that were not cohabiting with other animals and did not have any history of tick infestation. The samples were provided by the Brazilian Agricultural Research Corporation (Embrapa), from its Temperate Zone Division, in Pelotas, Rio Grande do Sul, Brazil. The desired confidence level was 99%.

For all the plates, spectrophotometric readings were taken until the positive control reached an OD of 1.0. For the positive control of the assay, bovine serum that was positive for *B. burgdorferi* was used. This was produced at the Parasitic Diseases Laboratory of the Animal Health project, under an agreement between UFRRJ and Embrapa. The production consisted of experimental inoculation of a healthy 50-day-old calf of 36 kg live weight, originating from the animal reproduction sector, Institute of Zootechnics, UFRRJ, using the methodology described by Ishikawa et al. (1997).

The statistical analyses were done using Fisher's exact test and the chi-square test, at the 5% confidence level.

Results

The antigen concentration of 20 µg/mL and the serum dilution of 1:400 and conjugate dilution of 1:5000 were the ones that gave the greatest difference (factor of 3.2 times) between the positive and negative serum samples. Therefore, these concentrations were used to modify the technique of Ishikawa et al. (1997).

The ELISA assay on 491 buffalo serum samples showed that 412 animals (83.9%) presented homologous antibodies of IgG class directed towards *B. burgdorferi* antigens. The animals in the Arari microregion of Marajó Island showed positivity of 81.7% (232/284), which did not differ statistically (p = 0.1488) from the positivity of 86.7% (180/207) observed in the microregions of Castanhal and Bragantina, located on the continental part of the state of Pará.

The frequencies of *B. burgdorferi* antibodies for each municipality are shown in Table 1. It was observed that the municipality of Santa Isabel showed the lowest frequency of positivity, but that this did not differ statistically from the municipality of Cachoeiras

Table 1. Frequency of homologous anti-*Borrelia burgdorferi* IgG antibodies in serum samples from buffaloes in the state of Pará, Brazil, as determined by means of the indirect ELISA test.

Municipality	Total number of samples	Frequency (%)
Cachoeiras do Arari	284	232 (81.7)[ab]
Castanhal	66	57 (86.4)[ac]
Santa Isabel	22	14 (63.6)[b]
Nova Timboteua	56	52 (92.9)[c]
Santarém Novo	63	57 (90.5)[ac]
Total	491	412 (83.9)

Values with the same letters do not differ from each other according to Fisher's exact test, at the 5% significance level.

do Arari (p = 0.0510). The percentages of positive findings in the other municipalities were statistically similar. The only exception was seen in Nova Timboteua, which presented a higher frequency than in Cachoeiras do Arari (p = 0.0472).

Discussion and Conclusions

In carrying out serological tests for *B. burgdorferi* among ruminants, especially when using crude antigen, the possibility of cross-reactions with *Borrelia theileri* should not be ruled out (ROGERS et al., 1999), especially since this agent is a spirochete that commonly affects cattle and horses, for which the main vector is the tick *Rhipicephalus (Boophilus) microplus* (SMITH; ROGERS, 1998). According to Barbour and Hayes (1986), the cross-reaction between these species can probably be partly explained by the fact that these spirochetes share a common flagellar antigen of 41 kDa, which is genus-specific to *Borrelia*. Ji et al. (1994) also reported the existence of cross-reactivity due to the 41 kDa antigen of *B. burgdorferi*, in an ELISA test done on cattle infected by *B. theileri*.

The lack of existence of a commercial buffalo anti-IgG conjugate and the close phylogenetic relationship between cattle and buffaloes, with strong homology between the immunoglobulins of the two species (CALLOW et al., 1976), justified the use of anti-cattle conjugate in the present study.

Use of highly specific recombinant proteins, such as those with molecular weights of 31 kDa (OspA), 34 kDa (OspB), 35 kDa (vlsE) and 110 kDa in the ELISA test, has improved the specificity of detection of antibodies against *B. burgdorferi* in humans, dogs, horses and cattle (GREENE et al., 1988; CAPUTA et al., 1991; MAGNARELLI et al., 1997, 2004, 2010). However, few data are available in relation to the performance and suitability of ELISA tests done with recombinant antigens. It is known that *B. burgdorferi* changes the expression of its surface antigens to avoid the host immune response (LIANG et al., 2004). In this respect, use of the whole antigen of *B. burgdorferi* gives higher sensitivity to the ELISA test (MAGNARELLI et al., 2004), and this is a suitable antigen for initial investigation and screening of a large number of serum samples.

Serological research among animals has shown that there is no significant relationship between the antibodies against *B. burgdorferi*, *Leptospira* sp. and *Treponema* sp. that causes a cross-reaction (MAGNARELLI et al., 1987; SOARES et al., 1999). High specificity (97%) has also been encountered among antibodies against *B. burgdorferi*, *Leptospira interrogans*, *Brucella* sp., *Anaplasma marginale* and *Anaplasma phagocytophilum* (MAGNARELLI et al., 2004).

In Brazil, seroepidemiological studies on domestic animals have already detected antibodies against *B. burgdorferi* in dogs (SOARES et al., 1999), horses (MADUREIRA et al., 2007; GALLO et al., 2009) and cattle (GUEDES JUNIOR et al., 2008). The frequency of seropositive buffaloes in the present work (83.9%) was markedly greater than the rate of 54.9% observed among cattle (GUEDES JUNIOR et al., 2008) and the 26.7% found among horses (GALO et al., 2009), in the same region. These differences in frequencies of seropositive animals may be connected with greater susceptibility among buffaloes and with the intrinsic immunological factors of each animal species. This draws attention to the possible importance of buffaloes in the epidemiology of borreliosis.

The high frequency of seropositive animals encountered, the presence of the vector *R.* (*Boophilus*) *microplus* and the possible presence of buffaloes infected with *Borrelia* spp. in the region, suggests that there is a cross-reaction between the G39/40 strain of *B. burgdorferi*, which is used as an antigen in the ELISA test, and the species or strain of *Borrelia* that possibly infects buffaloes in the state of Pará.

It is known that cattle are susceptible to infection by *B. theileri*, *B. burgdorferi* and *Borrelia coriaceae*. However, in Brazil, infection due to *B. coriaceae* still has not been detected, and there has also not been any successful isolation of *B. burgdorferi*. Recently, Mantovani (2010) suggested that *B. burgdorferi lato sensu* was a microorganism of atypical morphology for which culturing in aerobic and anaerobic media (including BSK) was impossible, and that it was observed in Brazilian patients with a disease similar to Lyme borreliosis. This affirmation was based on amplification and sequencing of a fragment of the gene *flgE*, which is responsible for synthesis of the flagellar hook of *B. burgdorferi*, and this author had also identified this spirochete in ticks on horses and cattle in the states of Espírito Santo and Rio de Janeiro, with 99% homology. Amplification and sequencing of the entire gene *flgE* and further studies need to be conducted with the aim of proving these indications.

This was the first seroepidemiological study on borreliosis in buffaloes in the northern region of Brazil, and it demonstrated that buffaloes in the region studied are highly seropositive for anti-*Borrelia burgdorferi* antigens.

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