

## FREQUENCY OF ANTIBODIES TO *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax* AND *Borrelia burgdorferi* IN CATTLE FROM THE NORTHEASTERN REGION OF THE STATE OF PARÁ, BRAZIL

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**ABSTRACT:**- GUEDES JUNIOR, D.S.; ARAÚJO, F.R.; SILVA, F.J.M.; RANGEL, C.P.; BARBOSA NETO, J.D.; FONSECA, A.H. **Frequency of antibodies to *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax* and *Borrelia burgdorferi* in cattle from the Northeastern region of the State of Pará, Brazil.** [Frequência de anticorpos para *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax* e *Borrelia burgdorferi* em bovinos do nordeste do Estado do Pará, Brasil]. *Revista Brasileira de Parasitologia Veterinária* v. 17, n. 2, p. 105-109, 2008. Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR 456, Km7, Seropédica, RJ 23890-000. E-mail: adivaldo@ufrj.br

Babesiosis, anaplasmosis, and trypanosomosis are relevant diseases, potentially causing morbidity in cattle, leading to economic losses. Borreliosis is import as a potential zoonosis. The objective of this study was to determine, by indirect enzyme-linked immunosorbent assay (ELISA), the frequency of seropositive cattle to *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax* and *Borrelia burgdorferi* in cattle from the Northeastern region of Pará, Brazil. Sera samples from 246 female adult cattle from municipalities of Castanhal and São Miguel do Guamá were used. Crude antigens ELISAs were used to detect antibodies to all agents, except to *A. marginale*, to which an indirect ELISA with recombinant major surface 1a protein (MSP1a) antigen was used. Overall frequencies of seropositive animals were: *B. bigemina* - 99.2%; *B. bovis* - 98.8%; *A. marginale* - 68.3%; *T. vivax* - 93.1% and *B. burgdorferi* - 54.9%. The frequencies of seropositive cattle to *B. bovis* and *B. bigemina* suggest a high rate of transmission of these organisms by tick in the studied region, which can be classified as enzootically stable to these hemoprotozoans. The low frequency of seropositive cattle to *A. marginale* may be attributed to a lower sensitivity of the recombinant antigen ELISA utilized or a distinct rate of inoculation of this rickettsia by ticks, as compared with *Babesia* sp. transmission. The high frequency of seropositive cattle to *T. vivax* indicates that this hemoprotozoan is prevalent in herds from the Northeastern region of Pará. The rate of animal that showed homologues antibodies to *B. burgdorferi* indicates the presence of the tickborne spirochaetal agent in the cattle population in the studied region.

**KEYWORDS:** *Babesia* sp., *Trypanosoma vivax*, *Anaplasma marginale*, *Borrelia* sp., antibodies.

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### RESUMO

A babesiose, a anaplasmose e a tripanossomose são enfermidades relevantes, potencialmente causadoras de morbidade em bovinos, levando a perdas econômicas. A borreliose assume importância como zoonose potencial. O objetivo desse estudo foi determinar, por meio do ensaio de imunoadsorção enzimática (ELISA), a frequência de anticorpos para *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax* e *Borrelia burgdorferi* em bovinos da região nordeste do Es-

tado do Pará, Brasil. Amostras de soro de 246 vacas dos municípios de Castanhal e São Miguel do Guamá foram usadas. ELISAs com antígeno bruto foram utilizados para detector anticorpos contra todos os agentes, exceto para *A. marginale*, para o qual um ELISA indireto com proteína principal de superfície 1a (MSP1a) foi usado. As frequências de bovinos soropositivos foram: *B. bigemina* – 99,2%; *B. bovis* – 98,8%; *A. marginale* – 68,3%; *T. vivax* – 93,1% and *B. burgdorferi* – 54,9%. As frequências de bovinos soropositivos para *B. bovis* e *B. bigemina* sugerem uma alta taxa de transmissão desses organismos por carrapatos, na região estudada, a qual pode ser classificada com sendo de estabilidade enzoótica para os hemoparasitos. A baixa frequência de bovinos soropositivos para *A. marginale* pode ser atribuída a uma menor sensibilidade do ELISA com antígeno recombinante, ou uma menor taxa de inoculação da riquetsia pelos carrapatos, quando comparada àquelas observadas para *Babesia* sp. A alta frequência de bovinos soropositivos para *T. vivax* indica que esse hemoprotozoário é prevalente em rebanhos do nordeste do Estado do Pará. O percentual de animais com anticorpos homólogos para *B. burgdorferi* indica a presença deste espiroquetídeo transmitido por carrapatos na população de bovinos da região estudada.

**PALAVRAS-CHAVE:** *Babesia* sp., *Trypanosoma vivax*, *Anaplasma marginale*, *Borrelia* sp., anticorpos.

## INTRODUCTION

Bovine hemoparasitic diseases cause important economic loss in tropical, subtropical, and humid equatorial areas, mainly due to the high morbidity and mortality in susceptible cattle herds. However, the most important economic constraint of hemoparasitic diseases to cattle production is on programs for genetic improvement of cattle, as imported *Bos taurus* cattle brought from temperate nations for breed improvement are highly susceptible to tick-borne diseases and often do not survive to become part of planned reproduction programs (MELENDEZ, 2000; KOCAN et al., 2003).

In Brazil, bovine babesiosis and anaplasmosis are caused by protozoans *Babesia bigemina* and *B. bovis* and by rickettsia *Anaplasma marginale*, respectively. During the acute infection, these pathogens cause anemia, low weight gain, reduction in milk production, and even mortality of susceptible animals (KOCAN et al., 2003; BOCK et al., 2004).

The distribution of *B. bigemina*, *B. bovis*, and *A. marginale* follows the dissemination of their vectors. In Brazil, the former agents are transmitted by *Rhipicephalus (Boophilus) microplus*, while the latter one, besides biological transmission by tick, can also be mechanically transmitted by hematophagous diptera (GUGLIELMONE, 1995). Epidemiological studies showed that, in Brazil, *B. bovis*, *B. bigemina* and *A. marginale* are endemic in several areas (ARAÚJO et al., 1998; SMITH et al., 2000; SOARES et al., 2000; SOUZA et al., 2000; MELO et al., 2001; ARAÚJO et al., 2005; BARROS et al., 2005, D'ANDREA et al., 2006). Other factor

that may influence the distribution of these pathogens is breed, as *B. indicus* cattle, because of tick resistance, are less likely to be infected by ticks. Also, management of the herds, with excessive use of acaricides and rotational grazing appears to be related to outbreaks of babesiosis and anaplasmosis, especially in dairy cattle (GUGLIELMONE, 1995; RIKHOTSO et al., 2005; D'ANDREA et al., 2006).

Other relevant hemoparasitic disease is tripanosomosis by *Trypanosoma vivax*. In South America, this hemoprotozoan is transmitted by hematophagous diptera, mainly tabanids (GARDINER, 1989; OTTE; ABUABARA, 1991; JONES; DÁVILA, 2001). In the Brazilian territory, *T. vivax* was first described in the State of Pará infecting water buffalo (*Bubalis bubalis*) (SHAW; LAINSON, 1972). Only 24 years later, *T. vivax* was reported in other parts of the country, in the Pantanal of Mato Grosso (SILVA et al., 1996) and one year later in the Pantanal of Mato Grosso do Sul (PAIVA et al., 2000). More recently, *T. vivax* was described in the semi-arid of the State of Paraíba (BATISTA et al., 2007). In Brazil, this hemoprotozoan was described causing fever, lethargy, weakness, anemia, diarrhea, abortion and even death of infected animals (SILVA et al., 1996; BATISTA et al., 2007).

*Borrelia theileri* is a worldwide tickborne spirochete that infects cattle and other mammals. In cattle, the infection can be characterized by fever, lethargy and anemia. This agent is specially important because *B. theileri* infection induces cross-reacting antibodies that could interfere with interpretation of seroepidemiologic studies of other bovine borreliae (ROGERS et al., 1999).

This study shows a serologic survey for antibodies to *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax* and homologous to *Borrelia burgdorferi* in cattle from the Northeastern region of the State of Pará and the discussion of epidemiological data, such as the endemicity status of the infectious agents.

## MATERIAL AND METHODS

Serum samples were obtained from 246 adult female cattle, selected by convenience, from the municipalities of Castanhal (65 beef cattle, Nelore breed) and São Miguel do Guamá (181 dairy cattle, crossbred), Northeastern region of State of Pará. These municipalities have 82,693 cattle, corresponding to 0.47% of the cattle population from the State of Pará (IBGE, 2006).

Serum samples were analyzed by indirect enzyme-linked immunosorbent assays (ELISAs) with crude antigens of *B. bigemina*, *B. bovis*, *T. vivax* (MADRUGA et al. 2000, 2001, 2006) and *B. burgdorferi* according to Ishikawa et al. (1997). *Anaplasma marginale* antibodies were detected by indirect ELISA, using recombinant MSP1a as antigen (ARAÚJO et al., 2005). Cutoffs were calculated for each ELISA plate, according to Frey et al. (1998), with 12 negative sera for each organism.

Statistical analyses were done by Qui-square or Fischer's exact tests, with a confidence level of 95%.

Table 1. Frequencies of seropositive cattle to *Babesia bigemina*, *B. bovis*, *Anaplasma marginale*, *Trypanosoma vivax* and *Borrelia burgdorferi* in cattle from the municipalities of Castanhal and São Miguel do Guamá, Northeastern region of Pará State, Brazil.

Organism	Castanhal (n = 65)		São Miguel do Guamá (n = 181)		Total (n = 246)	
	Positive	(%)	Positive	(%)	Positive	(%)
<i>Babesia bigemina</i>	64	(98.5)	180	(99.4)	244	(99.2)
<i>B. bovis</i>	62	(95.4)	181	(100)	243	(98.8)
<i>Anaplasma marginale</i>	43	(66.2)	125	(69.1)	168	(68.3)
<i>Trypanosoma vivax</i>	54	(83.0)	175	(96.7)	229	(93.1)
<i>Borrelia burgdorferi</i>	24	(36.9)	111	(61.3)	135	(54.9)

n = Number of samples.

## RESULTS AND DISCUSSION

From the 246 serum samples analyzed, 244 (99.2%), 243 (98.8%), 168 (68.3%), and 229 (93.1%) were positive for antibodies to *B. bigemina*, *B. bovis*, *A. marginale*, and *T. vivax*, respectively (Table 1) and, 135 (54.9%) were positive for homologous antibodies to *B. Burgdorferi*.

Frequencies of seropositive animals to *B. bovis* and *B. bigemina* were significantly higher ( $P < 0.001$ ) than those to *A. marginale* or *T. vivax*. Also, frequencies of seropositive cattle to *T. vivax* were significantly higher than that to *A. marginale* ( $P < 0.001$ ).

Differences in the frequencies of antibodies between Castanhal and São Miguel do Guamá were significant only for antibodies to *B. bovis* ( $P = 0.018$ ) and *T. vivax* ( $P < 0.001$ ), whose frequencies were higher in São Miguel do Guamá, were cross-bred dairy cattle were sampled. The effect of climatic conditions possibly is not related to these differences, as both municipalities are contiguous, and share the same meteorological conditions.

Frequencies of seropositive cattle to *B. bovis* and *B. bigemina* suggest a high rate of transmission of these organisms by tick in the studied region, which can be classified as enzootically stable to these hemoprotozoans (MAHONEY; ROSS 1972). This is suggestive that the risk of outbreaks of babesiosis is low and vaccination of native herds is not necessary.

The significantly lower frequency of antibodies to *A. marginale* may be attributed to a low rate of transmission by ticks or diptera, but this is hard to explain, in face of the high frequencies of antibodies to *Babesia* sp. Another possibility is a lower sensitivity of the recombinant MSP1a ELISA. If so, this is not in agreement with Araújo et al. (2005), who found a sensitivity of 99% for this test, with sera from naturally-infected cattle, positive for antibodies to *A. marginale* by IFAT. Silva et al. (2006) did not find differences in the performances of ELISAs with crude antigen and with MSP5 as antigens to detect antibodies to *A. marginale*.

The high frequency of antibodies to *T. vivax* is suggestive of an efficient mechanical transmission of this hemoprotozoan by hematophagous diptera (mainly tabanids), as biological vectors are not found in South America (GARDINER et al., 1989; OTTE; ABUABARA, 1991; JONES; DÁVILA, 2001). In Brazil, the first record of *T. vivax* was done in the State of

Pará, in water buffalo (SHAW; LAINSON, 1972). A recent epidemiological survey showed the presence of specific antibodies to this hemoprotozoan in different regions of the State of Pará (MADRUGA et al., 2006). These data and those found in this study indicate that *T. vivax* is possibly widespread in the State of Pará.

In Brazil, *T. vivax* has been involved with chronic and asymptomatic infection (VENTURA et al., 2001; DÁVILA et al., 2003), but clinical cases have been recorded, with cattle showing anemia, weight loss, hypoglycemia, and nervous signs (BATISTA et al., 2007). In this context, studies to evaluate the economic impact of this hemoprotozoan in the State of Pará are necessary.

The subclinical aspects of *B. theileri* infection described by Rogers et al. (1999) were observed in this study. The occurrence of the Lyme disease like syndrome referred by Mantovani et al. (2007) in Brazil, justify the seroepidemiological study in cattle, because of the cross-reacting antibodies between *Borrelia theileri* and other species of *Borrelia* genus (ROGERS et al., 1999). The rate of cattle that showed homologous antibodies to *B. burgdorferi* indicates the presence of the tickborne spirochaetal agent in the herds evaluated. More studies should be done to evaluate the risk to human of this *Borrelia* species present in cattle.

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Received on October 30, 2007.

Accepted for publication on June 24, 2008.