Full Article

Rev. Bras. Parasitol. Vet., Jaboticabal, v. 22, n. 2, p. 207-213, abr.-jun. 2013
ISSN 0103-846X (impresso) / ISSN 1984-2961 (eletrônico)

Seroprevalence and risk factors for cattle anaplasmosis, babesiosis, and trypanosomiasis in a Brazilian semiarid region

Soroprevalência e fatores de risco para anaplasmose, babesiose e tripanosomíase bovina em uma região semiárida do Brasil

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Received June 12, 2012
Accepted February 18, 2013

Abstract

The seroprevalence of *Anaplasma marginale, Babesia bigemina, Babesia bovis* and *Trypanosoma vivax* and the risk factors for these infections were investigated in 509 cows on 37 farms in the semiarid region of Paraíba, northeastern Brazil. Cow serum samples were tested by means of immunofluorescence assay (IFA) against each specific antigen. The mean seroprevalence values per farm were 15.0% (range: 0-75%) for *A. marginale,* 9.5% (range: 0-40%) for *B. bigemina* and 26.9% (range: 0-73.7%) for *B. bovis.* All cows tested negative for *T. vivax.* Higher prevalence for *A. marginale* was significantly associated with less frequent acaricide spraying per year and with higher use of injectable antihelminthics. Presence of cows positive for *B. bigemina* was significantly associated with acaricide use and with presence of horse flies on the farm. Both occurrence and higher prevalence of *B. bovis* were significantly associated with recent observations of ticks on cattle. Overall, the present results indicate that the region investigated is an enzootically unstable area for *A. marginale, B. bigemina* and *B. bovis*, since most animals were seronegative to at least one agent.

Keywords: *Anaplasma marginale, Babesia bigemina, Babesia bovis,* cattle, risk factors, Brazil.

Resumo

A soroprevalência de *Anaplasma marginale, Babesia bigemina, Babesia bovis* e *Trypanosoma vivax,* assim como os fatores de risco para estas infecções, foram investigadas em 37 fazendas (total de 509 vacas) da região semiárida da Paraíba, nordeste do Brasil. A presença de anticorpos nos soros dos animais foi detectada pela técnica de imunofluorescência indireta, utilizando antígenos específicos. Os valores médios de soroprevalência por fazenda foram 15,0% (0-75%) para *A. marginale,* 9,5% (0-40%) para *B. bigemina,* e 26,9% (0-73,7%) para *B. bovis.* Todas as vacas foram soronegativas para *T. vivax.* As maiores prevalências de *A. marginale* foram significativamente associadas com menor uso de carrapaticidas por ano e com uso mais frequente de antihelmínticos injetáveis. A soroprevalência de *B. bigemina* foi significativamente associada com o uso de carrapaticidas, e com a presença de mutucas na fazenda. Tanto a ocorrência como a maior soroprevalência para *B. bovis* nas fazendas foram significativamente associadas com a presença recente de carrapatos nos bovinos. No geral, os resultados indicam que as fazendas amostradas estão situadas em área de instabilidade enzoótica para *A. marginale, B. bigemina,* e *B. bovis,* uma vez que a maioria dos animais foi soronegativa para pelo menos um dos agentes.

Palavras-chave: *Anaplasma marginale, Babesia bigemina, Babesia bovis,* bovino, fatores de risco, Brasil.

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Introduction

With over 200 million cattle, Brazil has the largest commercial herd in the world, and most of these animals are exposed to the cattle tick, *Rhipicephalus (Boophilus) microplus*. Most of the time, *R.* (*B.*) *microplus* is the sole tick species infesting cattle in Brazil (ARAGÃO, 1936; CAMPOS PEREIRA et al., 2008). This tick species is a primary vector of the bacterium *Anaplasma marginale* and the protozoa *Babesia bigemina* and *Babesia bovis*, which may contribute towards the generally high prevalence of such tick-borne agents throughout the country. However, occurrences of diseases caused by these agents are generally dependent on the climatic conditions of each region. In most of the country, cattle babesiosis and anaplasmosis occur endemically because environmental conditions provide moderate to high *R.* (*B.*) *microplus* burdens infesting pastured cattle throughout the year. In this case, cattle generally become infected by *A. marginale, B. bigemina* and *B. bovis* during the first weeks or months after birth, and then remain infected (seropositive) without suffering severe disease for the rest of their lives (RIBEIRO; REIS, 1981; MADRUGA et al., 1984, 1985; PATARROYO et al., 1987; SANTOS et al., 2001). On the other hand, environmental conditions in some areas are not suitable for *R.* (*B.*) *microplus* throughout the year, such as in southern Brazil (and Uruguay), where mean temperatures below 15 °C inhibit tick development during the autumn-winter months. Consequently, many animals do not come into contact with infected ticks during their first months, and when they first become infected later on, severe disease with high lethality is a common feature (NARI; SOLARI, 1991; MARTINS et al., 1994; GUGLIELMONE, 1995; MARANA et al., 2009).

The African agent for surra, *Trypanosoma vivax,* which was first reported in South America in the early 20th century, is considered to be mechanically transmitted by biting flies, such as tabanids and *Stomoxys calcitrans* (HOARE, 1972). These flies have also been incriminated as mechanical vectors for *A. marginale* (HAWKINS et al., 1982; KESSLER, 2001). In Brazil, *T. vivax* has been sporadically reported infecting ruminants, as recently reported in three outbreaks affecting cattle in the semiarid region of the State of Paraíba (BATISTA et al., 2007, 2008).

The state of Paraíba, located in the northeastern region of Brazil, is characterized by warm weather throughout the year. The state is geographically subdivided into the following four major regions, based mostly on vegetation type and rainfall: (i) Zona da Mata (Atlantic forest), (ii) Agreste, (iii) Cariri, and (iv) Sertão (Figure 1). The Zona da Mata and Agreste have relatively higher rainfall regimes (CABRERA; WILLINK, 1973). Both Cariri and Sertão (the semiarid region) are typically within the Caatinga biome, which encompasses an area of 900,000 km^2 (11% of Brazilian territory) and is the only major biome that occurs exclusively in Brazil. Caatinga is xeric shrubland and thorn forest, which consists primarily of small, thorny trees that shed their leaves seasonally. Cacti, thick-stemmed plants, thorny brush and arid-adapted grasses make up the ground layer; however, during the dry periods there is no ground foliage or undergrowth (ANDRADE-LIMA, 1981). The weather is characterized by a hot and semiarid climate, with temperatures averaging 27 °C, and the mean annual rainfall is typically ≈500 mm. There are typically two seasons: a rainy season from February to May, and a long drought period from June to January. However, occurrences of droughts sometimes lasting for longer than one year is also a characteristic of the region (BATISTA et al., 2007). Indeed, this usually long drought period has important implications that are yet to be evaluated under natural conditions, for the vectors of *A. marginale* and *Babesia* spp. in relation to cattle raised in the Caatinga biome.

During the last few decades, dairy cattle have become significantly important within animal husbandry in Paraíba. Except for the Zona da Mata region (where sugarcane crops prevail), small cattle-raising farms are widespread in the Agreste, Cariri and Sertão regions. Whereas cultivated grasses (mostly *Brachiaria* spp.) are the basis for Agreste livestock, cattle are usually reared extensively on native Caatinga in most of the Cariri and Sertão farms. Until very recently, cattle babesiosis or anaplasmosis had remained unreported in the Caatinga biome of the state of Paraíba. A recent study by our group described 24 outbreaks of cattle anaplasmosis and/or babesiosis on cattle farms in the Sertão region of that state (COSTA et al., 2011). These outbreaks mostly affected cows, with a 36% mean lethality rate (range: 0-100%), thus indicating that many of the affected cows were not immune, i.e. they had not previously been infected by *Anaplasma* or *Babesia* agents. Here, for the first time, we investigated the seroprevalence of cattle for *A. marginale, B. bigemina, B. bovis* and *T. vivax* on farms in the semiarid region of Paraíba, as well as the risk factors for these agents.

Materials and Methods

From July 2009 to July 2010, blood samples were collected from cattle on 37 small farms in the state of Paraíba, of which 25 were in the Sertão, 9 in the Cariri and 3 in the Agreste region (Figure 1). A total of 509 blood samples were obtained. On each farm, 5 to 30 cows were sampled. Only small farms with herd sizes ranging from 8 to 180 cattle were investigated. During farm visits, a standard questionnaire was applied to the farm owner or manager in order to obtain information for studying the risk factors. The independent variables studied are shown in Table 1.

Each cow serum sample was tested by means of immunofluorescence assay (IFA) against antigens of *A. marginale* strain UFMG1*, B. bigemina* strain BbigMG*, B. bovis* strain BbovMG and *T. vivax* strain IgarapeMG*,* as previously described (BASTOS et al., 2010; CUGLOVICI et al., 2010). For the first three agents, the antigens consisted of parasitized erythrocytes obtained from splenectomized calves that had been experimentally inoculated (IICA, 1987). The antigens for *T. vivax* consisted of fixed trypomastigotes, obtained from an experimentally-infected splenectomized goat (CUGLOVICI et al., 2010). Serum samples were considered positive if they displayed a specific reaction to one of the four antigens, at the 1:80 serum dilution. In each reaction, we used appropriate negative and positive control sera from experimentally or naturally-infected cattle with each of the agents (COSTA-JÚNIOR et al., 2006; BASTOS et al., 2010; CUGLOVICI et al., 2010).

The proportions of the cows that were serologically positive for *A. marginale, B. bigemina* or *B. bovis* were calculated separately for

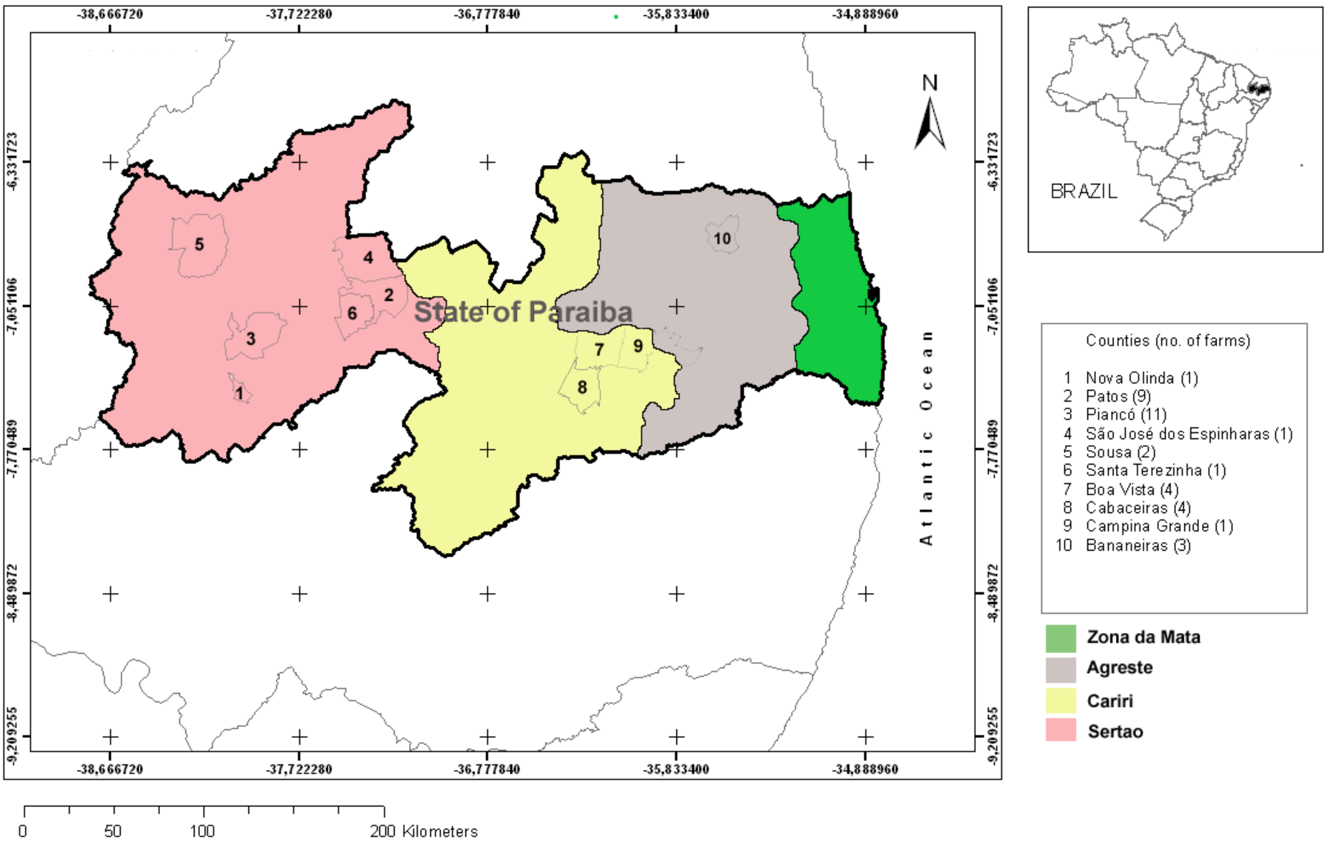

Figure 1. Numbers of farms per county (municipality) according to the geographic regions of the state of Paraíba, northeastern Brazil.

each farm. For data analysis, logistic regression was performed to construct multivariate models that could explain the dependent variables (cows serologically positive for *A. marginale, B. bigemina* or *B. bovis*) as a function of the 24 independent variables, dichotomously (absence = 0; presence = 1) (Table 1). The serological results from each farm were analyzed at two separate dichotomous levels: firstly, with the presence or absence of serologically positive cows (no positive cow = 0; at least one positive cow on the farm = 1); and secondly, as the proportion of positive cows on the farm (less than 25% of cows positive = 0; more than 25% of cows positive = 1). The independent variables were subjected to univariate analysis and those with a statistical association (here considered to be $P < 0.20$, using the chi-square test, for this first analysis) were tested in the multivariate model by means of the stepwise forward method. The variables were included in the multivariate model if they displayed statistical significance of $P < 0.05$, considering also the significance level of the final model. All analyses were performed using SPSS for Windows (SPSS, 1999).

Results

Among the 509 cows sampled, 211 (41.5%) were seropositive for at least one agent. Simple infection was observed in 170 cows (33.4%), of which 53 were positive solely for *A. marginale,* 33 solely for *B. bigemina* and 84 solely for *B. bovis*; dual infection was observed in 34 cows (6.7%), of which 24 for both *A. marginale* and *B. bovis,* and 10 for both *B. bigemina* and *B. bovis*; triple infection, namely, seropositivity for *A. marginale, B. bigemina* and *B. bovis,* was detected in only 7 cows (1.4%). All the cows were seronegative for *T. vivax.* Overall seroprevalence values according to geographic regions are shown in Table 2. The number of cows sampled per farm ranged from 5 to 30 (mean: 13.8 ± 6.2). The mean seroprevalence values per farm were 15.0 ± 18.1% (range: 0-75%) for *A. marginale,* 9.5 ± 12.5% (range: 0-40%) for *B. bigemina* and 26.9 ± 24.1% (range: 0-73.7%) for *B. bovis.* Among the 37 farms sampled, all the cows were negative for *A. marginale, B. bigemina* and *B. bovis*, respectively, on 14 (37.8%), 18 (48.6%) and 7 (18.9%) of the farms. Seropositivity prevalences above 25% for *A. marginale, B. bigemina* and *B. bovis*, respectively, were observed on 10 (27.0%), 4 (10.8%) and 17 (45.9%) of the farms.

By means of univariate analysis, the presence of at least one cow serologically positive for *A. marginale* was statistically coupled ($P < 0.20$) with variable nos. 3, 9, 18, 20 and 23 (Table 1). However, when these independent variables were subjected to multivariate analysis, none was significant. Univariate analysis showed that situations with more than 25% of the cows serologically positive for *A. marginale* were statistically coupled ($P < 0.20$) with variable nos. 2, 3, 4, 12, 13, 19 and 20. When these independent variables were subjected to multivariate analysis, only use of less than four acaricide sprayings per year (no. 13), and more than two

Table 1. Dichotomous categories of each independent variable used in the statistical analysis of seropositivity of cows for *Anaplasma marginale, Babesia bigemina* and *Babesia bovis* in the state of Paraíba, Brazil.

Nº.	Independent variables	Code 0*	Code 1*
1	Region	Sertão [25]	Cariri or Agreste [12]
2	Farm size	≤64 ha [21]	>64 ha [16]
3	Herd size	≤45 cattle [19]	>45 cattle [18]
4	No. of cows on the farm	≤15 [24]	>15 [13]
5	Predominant cattle breed	Crossbred or Zebu [31]	European [6]
6	Exploitation type	Mixed or beef [8]	Dairy [29]
7	Predominant type of pasture	Native [19]	Cultivate [18]
8	Artificial irrigation of pastures	No [25]	Yes [12]
9	Recent observation of ticks on cattle#	No [6]	Yes [31]
10	Ticks on cattle throughout the year	No [23]	Yes [14]
11	Tick control with chemical acaricides on cattle	No [7]	Yes [30]
12	Acaricide method of application	Others [11]	Spraying [26]
13	No. of acaricide sprayings per year	<4 [12]	≥4 [25]
14	Presence of horse flies (Tabanidae)	No [10]	Yes [27]
15	Presence of mosquitoes	No [10]	Yes [27]
16	Presence of horn flies	Part of the year [32]	All year long [5]
17	Use of chemical control for horn flies	No [27]	Yes [10]
18	Use of injectable antihelminthics	No [6]	Yes [31]
19	No. of injectable antihelminthics per year	≤2 [26]	>2 [11]
20	Use of rabies vaccination in the last 2 years	No [6]	Yes [31]
21	Use of disposable needles individually	No [16]	Yes [21]
22	Recent introduction of cattle into the herd	No [21]	Yes [16]
23	No. of animals introduced	≤2 [31]	>2 [6]
24	Tick infestation on introduced animals	No [26]	Yes [11]

*numbers between brackets refer to the number of farms with the given condition for each variable. #all ticks found on cattle were taxonomically identified as *Rhipicephalus (Boophilus) microplus* during farm visits; Nº - Number.

Table 2. Results from immunofluorescence assay (IFA) performed with *Anaplasma marginale, Babesia bigemina* and *Babesia bovis* antigens on blood serum samples from cows in three geographic regions of the state of Paraíba, northeastern Brazil.

Region	Nº. of cows tested	Nº. cows serologically positive [% (confidence level)]		
		A. marginale	*B. bigemina*	*B. bovis*
Sertão	342	56 [16.4 (12.6-20.7)]	32 [9.3 (6.5-12.9)]	75 [21.9 (17.6-26.7)]
Cariri	115	25 [21.7 (14.6-30.4)]	11 [9.6 (4.9-16.5)]	39 [33.9 (25.3-43.3)]
Agreste	52	3 [5.8 (1.2-15.9)]	7 [13.5 (5.6-25.8)]	11 [21.1 (11.1-34.7)]
Total	509	84 [16.5 (13.4-20.0)]	50 [9.8 (7.4-12.6)]	125 [24.5 (20.9-28.5)]

Nº - Number.

applications of injectable antihelminthics per year (no. 19) were significantly associated ($P < 0.05$) (Table 3).

Through univariate analysis, the presence of at least one cow serologically positive for *B. bigemina* was statistically coupled ($P < 0.20$) with variable nos. 12, 14, 18 and 23. When these independent variables were subjected to multivariate analysis, only use of spraying for acaricide application (no. 12) and presence of horse flies on the farm (no. 14) were significantly associated ($P < 0.05$) (Table 3). Univariate analysis showed that no independent variable was associated at the $P < 0.20$ significance level with prevalence of *B. bigemina* higher than 25%.

By means of univariate analysis, the presence of at least one cow serologically positive for *B. bovis* was statistically coupled ($P < 0.20$) with variable nos. 6, 8, 9, 11, 12 and 13. When these independent variables were subjected to multivariate analysis, only recent observation of ticks on cattle (No. 9) was significantly associated ($P < 0.05$) (Table 3). Univariate analysis showed that situations with more than 25% of the cows serologically positive for *B. bovis* were statistically coupled ($P < 0.20$) with variable nos. 4, 9, 11 and 13. When these independent variables were subjected to multivariate analysis, only recent observation of ticks on cattle (no. 9) was significantly associated ($P < 0.05$) (Table 3).

Discussion

Based upon the classical epidemiological conceptual model, the risk of babesiosis and anaplasmosis outbreaks can be indirectly measured by determining the proportion of cattle of a known age that have anti-*Babesia* spp. and anti-*A. marginale* antibodies (MAHONEY; ROSS, 1972; ALONSO et al., 1992; GUGLIELMONE et al., 1997). In this case, it is desirable for

Table 3. Results from multivariate analyses between cows that were serologically positive for *Anaplasma marginale, Babesia bigemina* or *Babesia bovis* and independent variables. Each dependent variable was analyzed with independent variables that were previously selected in univariate analyses, considering $P < 0.20$.

Independent variables	Nº. of farms	Nº. of farms positive (%)	Odds ratio	95% CI (odds ratio)	*P*-value
*Farms with >25% of cows serologically positive for A. marginale***					
Nº. of acaricide sprayings on cattle per year					
<4	12	6 (50.0)			
≥4	25	4 (16.0)	14.3	1.5 – 138.0	0.021
Nº. of injectable antihelminthics per year					
≤2	26	5 (19.2)			
>2	11	5 (45.5)	10.9	1.1 – 108.7	0.041
*Farms with at least one cow serologically positive for B. bigemina***					
Acaricide method of application					
Others	11	3 (27.3)			
Spraying	26	16 (61.5)	7.5	1.3 – 42.5	0.023
Presence of horse flies (Tabanidae) on the farm					
No	10	2 (20.0)			
Yes	27	17 (63.0)	11.4	1.7 – 76.2	0.012
*Farms with at least one cow serologically positive for B. bovis***					
Recent observation of ticks on cattle					
No	6	2 (33.3)			
Yes	31	28 (90.3)	18.7	2.3 – 148.4	0.006
*Farms with >25% of cows serologically positive for B. bovis***					
Recent observation of ticks on cattle					
No	6	0 (0.0)			
Yes	31	17 (54.8)	*	*	0.022

*It was not possible to calculate the odds ratio because one of the values was zero. **dependent variable; Nº - number; CI - confidence interval.

primary exposure to infected vectors to occur among calves up to 7-9 months of age, when they are naturally resistant to clinical effects and develop immunity lasting for at least two years. Cattle herds under this condition are considered to present enzootic stability with regard to babesiosis or anaplasmosis (MAHONEY; ROSS, 1972). On the other hand, on farms where primary exposure to infected vectors does not occur among calves under 9 months of age, cattle herds are considered to present enzootic instability with regard to babesiosis or anaplasmosis because older seronegative cattle become highly susceptible to the clinical effects of babesiosis or anaplasmosis, and consequently, the farm is at risk of outbreaks of these diseases (MAHONEY; ROSS, 1972).

Overall, our results indicate that the farms sampled in the present study may be in a situation of enzootic instability for *A. marginale, B. bigemina* or *B. bovis*, since most of the animals were seronegative for at least one agent. This condition is corroborated by recent outbreaks of anaplasmosis and babesiosis reported in 24 farms in the Sertão region of Paraíba (COSTA et al., 2011). These outbreaks, which occurred from 2007 to 2009, were associated with atypically higher rainfall that favored higher vector abundance and, consequently, higher exposure of susceptible animals to infected vectors: both ticks (in the case of *Babesia* spp. and *A. marginale*) and biting flies (in the case of *A. marginale*) (COSTA et al., 2011).

The cattle seroprevalence values were two to three times higher for *B. bovis* than for *B. bigemina*, although both agents were generally found in low percentages in the study region, with most of the cows being seronegative. Mahoney and Ross (1972) reported that if tick populations are suppressed either by natural or by artificial conditions, cattle might not be exposed to *Babesia*-infected ticks because their numbers under natural conditions are extremely low. This should explain the overall low seroprevalence values found in the present study, in which only 14 farms (37.8%) reported tick infestations throughout the year (Table 1). In addition, since primary *B. bovis* infection tends to last for a much longer period than primary *B. bigemina* infection (MAHONEY; ROSS, 1972), higher seroprevalence for *B. bovis* should be expected in marginal areas like the semiarid region of Paraíba, where cattle exposure to *Babesia* spp-infected ticks might not be frequent. Therefore, animals with primary infection due to *B. bigemina* may become seronegative before being reinfested by *B. bigemina*-infected ticks.

Three outbreaks of cattle trypanosomiasis due to *T. vivax* were recently reported on different farms in the Sertão region of Paraíba between 2002 and 2006 (BATISTA et al., 2007, 2008). While the origin of the first outbreak in 2002 remains unknown, the subsequent two outbreaks (2005 and 2006) were associated with cattle movements among these farms (BATISTA et al., 2008). These outbreaks occurred in the north of the state of Paraíba, approximately 70 km from the nearest farms sampled in the present study, which were located in the municipalities of Sousa and São José do Espinharas. We did not find any serological evidence of

T. vivax infection in the cattle of the present study, which led us to conclude that this parasite is not widespread in the state. It might have encountered ecological barriers against spreading, such as absence of vectors during the typically long drought period.

Higher farm seroprevalence values (>25%) for *A. marginale* were significantly associated with less frequent (<4) acaricide spraying per year. This may have been related not only to higher cattle exposure to ticks, but also to higher exposure to biting flies, since most commercial acaricides are synthetic pyrethroids, which are potent fly repellents (MENCKE, 2006). Bloodsucking flies may play an important role in mechanical transmission of *A. marginale* in the region. Besides the fact that experimental transmission of *A. marginale* by tabanids has been successfully demonstrated, it has also been observed that horse flies may remain mechanically infective for at least two hours after a blood meal on an infected animal (HAWKINS et al., 1982). Indeed, some authors have considered that mechanical transmission of *A. marginale* by biting flies is even more important than biological transmission by ticks under certain conditions, especially because of the lack of transovarial transmission of *A. marginale* in *R.* (*B.*) *microplus* ticks (UILENBERG, 1970; RIBEIRO; LIMA, 1996), as well as the inability of some strains of *A. marginale* to infect ticks, including *R.* (*B.*) *microplus* (GONÇALVES RUIZ et al., 2005). Higher seroprevalences of *A. marginale* have also been associated with several (>2) applications of injectable antihelminthics per year. This observation might be related to iatrogenic transmission of *A. marginale*, which has been reported to be an important transmission route for this agent among cattle in Brazil (KESSLER, 2001).

The presence of at least one cow serologically positive for *B. bigemina* on the farm was significantly associated with use of spraying for acaricide application. While this association may seem unexpected at first glance, it might be inferred that farms treating their cattle should have higher tick infestations. In Brazil, the vast majority of farmers use manual backpack sprayers for applying acaricides on cattle, which usually results in incorrect application under field conditions, and consequently, inefficient tick control (AMARAL et al., 2011). In the present study, all the farmers who reported that they sprayed their cattle also reported that they used backpack sprayers (data not shown). Therefore, inefficient application and poor tick control does not suppress the chances of cattle being exposed to *B. bigemina*-infected ticks. The presence of *B. bigemina*-infected cows was also associated with the presence of horse flies on the farm. Since *Boophilus* ticks are the only known vectors in Brazil of *B. bigemina* (GUGLIELMONE, 1995), it could be inferred that the noticeable presence of tabanids on the farms may have been related to suitable environmental conditions not just for horse flies but also for ticks, since humid lowlands that would provide breeding sites for tabanids may also favor pastures with suitable vegetation coverage for tick reproduction. Finally, both occurrence and higher seroprevalence of *B. bovis* on the farms were significantly associated with recent observation of ticks on cattle, which was an expected association because *Boophilus* ticks are the only known vectors in Brazil of *B. bovis* (GUGLIELMONE, 1995)

In conclusion, the present study shows that in a region of the state of Paraíba where a semiarid and hot climate prevails, bovine babesiosis and anaplasmosis occur under conditions of enzootic instability (≤75% seroprevalence). Although the sampling for our survey, which covered 37 cattle farms that were randomly selected according to logistic reasons, does not statistically represent the whole diversity of situations in the state, the results obtained from these 37 farms were very consistent regarding the serological results; i.e., with a few exceptions, most animals on the farms were seronegative for *B. bigemina, B. bovis* and *A. marginale.* The characteristic weather (long drought period) and vegetation type (Caatinga) of the region seem to be related to this enzootic instability, similarly to the conditions reported for the semiarid Chaco biome in northern Argentina (GUGLIELMONE et al., 1997). Thus, adoption of yearly vaccination of calves may be an appropriate practice for preventing babesiosis or anaplasmosis outbreaks on farms within the Caatinga biome of the state of Paraíba, just like it has been adopted in southern Brazil, where enzootic instability also prevails, but due to another reason: the extremely low autumn-winter temperatures.

Acknowledgements

We thank Alberto L. Rodrigues for his technical support during field work. This work was supported by the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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