

Prevalence of equine Piroplasmosis and its association with tick infestation in the State of São Paulo, Brazil

Prevalência da Piroplasmose equina e sua associação com infestação por carrapatos no Estado de São Paulo

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Abstract

Serum samples were collected from 582 horses from 40 stud farms in the State of São Paulo and tick (Acari: Ixodidae) infestations were evaluated on them. Serum samples were subjected to the complement fixation test (CFT) and a competitive inhibition ELISA (cELISA) for *Babesia caballi* and *Theileria equi*. Logistic regression analyses were performed to construct multivariate models that could explain the dependent variable (horses positive for *B. caballi* or *T. equi*) as a function of the independent variables (presence or abundance of each one of the tick species found on the farms). A higher overall prevalence of *B. caballi* (54.1%) than of *T. equi* (21.6%) was found by the two tests. The ticks *Dermacentor nitens* Neumann, 1897, *Amblyomma cajennense* (Fabricius, 1787) and *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) were present on horses on 38 (95%), 20 (50%), and 4 (10%) farms, respectively. Infestations by *D. nitens* were statistically associated with *B. caballi*-positive horses on the farms by either the CFT or cELISA. Infestations by *A. cajennense* were statistically associated with *T. equi*-positive horses on the farms by either CFT or cELISA.

Keywords: *Babesia caballi*, *Theileria equi*, Brazil, horse, tick.

Resumo

Amostras de soro sanguíneo foram coletadas de 582 equinos de 40 haras no estado de São Paulo, onde as infestações por carrapatos foram avaliadas nos animais. Os soros foram testados por reação de fixação do complemento (RFC) e ELISA competitivo por inibição (cELISA) com antígenos de *Babesia caballi* e *Theileria equi*. Análises de regressão logística foram realizadas para construir modelos multivariados que pudessem explicar as variáveis dependentes (equinos positivos para *B. caballi* ou *T. equi*) em função de variáveis independentes (presença e abundância de cada uma das espécies de carrapatos encontradas nos equinos dos haras). Em geral, os dois testes sorológicos indicaram uma prevalência maior para *B. caballi* (54,1%) do que para *T. equi* (21,6%). Os carrapatos *Dermacentor nitens* Neumann, 1897, *Amblyomma cajennense* (Fabricius, 1787) e *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) estiveram presentes em equinos de 38 (95%), 20 (50%) e 4 (10%) haras, respectivamente. As infestações por *D. nitens* estiveram estatisticamente associadas com equinos positivos para *B. caballi* tanto pela RFC como pelo cELISA. As infestações por *A. cajennense* estiveram estatisticamente associadas com equinos soropositivos para *T. equi*, tanto pela RFC como pelo cELISA.

Palavras-chave: *Babesia caballi*, *Theileria equi*, Brasil, cavalo, carrapato.

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Introduction

Equine piroplasmosis is a protozoan disease caused by two agents in Brazil: *Babesia caballi* and *Theileria equi* (BARBOSA et al., 1995; HEUCHERT et al., 1999; KERBER et al., 1999; CANOLA et al., 2007; HEIM et al. 2007; BALDANI et al., 2008). The severity of clinical signs is variable, and in many cases spontaneous recovery may occur without obvious clinical signs. This is especially true in endemic areas where foals become infected while they are protected by maternal antibodies and other non-specific factors, which render them partially resistant to challenge (RIBEIRO et al., 1995). Maternal antibodies are usually undetectable 3 to 5 months after birth (ALLSOPP et al., 2007; KUMAR et al., 2008). Therefore, foals undergo a non-apparent infection but there is some evidence that stress factors may lead to relapsing clinical episodes later in life (DE WAAL, 1995; FRIEDHOFF, 1988).

Equine piroplasmosis is an important disease of equids and it has a specific impact on international trade. Horses infected with equine piroplasmosis remain carriers for long periods and act as source of infection for ticks. *Theileria equi* can also be transmitted iatrogenically by the reuse of needles (TENTER; FRIEDHOFF, 1986;). Stress, such as after intensive training or transport, may lead to recrudescence parasitaemia in carrier animals, influencing the performance of sporting horses. Reproductive failure of mares, as a result of intra-uterine infection of the fetus, particularly with *T. equi*, is quite common in some countries (CORREA et al., 1978; DE WAAL, 1995). Elimination of *T. equi* infections by chemotherapy is unsatisfactory. To prevent the introduction into disease-free countries, very strict international import and export regulations are applied and movement of horses is only permitted if they are deemed to be free of infection, *i.e.*, a negative result on the indirect immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) (OFFICE INTERNATIONAL DES EPIZOOTIES, 2005). Until the beginning of this decade, the complement fixation test (CFT) was also accepted for international trade of horses (USDA, 1997; DE WAAL, 2000). Nowadays, this test is still widely used for other purposes (OGUNREMI et al., 2007).

Serological studies concerning infection by *B. caballi* and *T. equi* have shown that these two agents are widely distributed in Brazil, with herd prevalence ranging from 1.4 to 100% (BARBOSA et al., 1995; BITTENCOURT et al., 1997; KERBER et al., 1999; RIBEIRO et al., 1999; XUAN et al., 2001; BOTTEON et al., 2002; BALDANI et al., 2004; 2007; COSTA PEREIRA et al., 2005; HEIM et al., 2007). These values have been shown to be affected by climatic conditions and farm management.

Studies have shown that three major tick species infest horses in Brazil: *Dermacentor nitens* (= *Anocentor nitens*), *Amblyomma cajennense*, and *Rhipicephalus (Boophilus) microplus* (FALCE, 1986; BARBOSA et al., 1995; HEUCHERT et al., 1999; LABRUNA et al., 2001; COSTA PEREIRA et al., 2005). Although horses are considered primary hosts for *D. nitens* and *A. cajennense*, they are not so for *R. (B.) microplus*, as their infestation by this tick is dependent on the presence of cattle grazing with horses in the same pasture (LABRUNA et al., 2001). On the American continent, *D. nitens* is the only known vector of

B. caballi (ROBY; ANTHONY, 1963) and in Brazil the prevalence of *B. caballi* mainly coincides with the distribution of this tick. Although *T. equi* is transmitted by different two- and three-host ticks in the Old World (FRIEDHOFF, 1988), information on natural tick vectors is still limited in the New World (STILLER; COAN, 1995). Dennig (1988) was unsuccessful in infecting *A. cajennense* and *D. nitens* with *T. equi*. On the other hand, the vector competence of *R. (B.) microplus* has been repeatedly demonstrated, with successful transtadial and intrastadial survival of *T. equi* in this tick (GUIMARÃES et al., 1998; STILLER et al., 2002; UETI et al., 2005; 2008). In fact, *T. equi*-infected horses have been reported in areas where *R. (B.) microplus* is the only tick found parasitizing horses, especially in southern Brazil (SOUZA et al., 2000). *Rhipicephalus (B.) microplus* is a one-host tick and the epidemiological significance of this tick vector remains under discussion, especially for highly endemic areas, since it is accepted that there is no transovarial transmission of *T. equi* in ticks.

Studies on risk factors could support the adoption of specific measures that would minimize the introduction or dispersion of the disease among farms. Moreover, such studies could indicate epidemiological evidence about the mechanisms by which the agent disseminates in the host population. Thus, the present work was conducted with the purpose of evaluating the prevalence of equine piroplasmosis and possible associations between ticks infesting horses and the presence of serologically positive horses in the State of São Paulo, with emphasis on *T. equi*.

Material and Methods

1. Farms

From December 1998 to March 1999, 40 stud farms were studied in the state of São Paulo, southeastern Brazil. The farm localities and number of farms per locality have been illustrated elsewhere (LABRUNA et al., 2001). All farms had horse breeding as the major livestock activity and the equine breeds among the 40 farms included Thoroughbred (8 farms), Quarter Horse (7), Mangalarga Paulista (5), Mangalarga Marchador (4), Andalusian (4), Lusitanian (4), Brasileiro de Hipismo (4), Arabian (3), and Morgan (1).

2. Farm visits

During visits to farms, blood samples were obtained in siliconized vacutainer tubes from all horses 6-24 months old, reared under grazing conditions. If any of these horses had left the farms for any reason during their lives, or if any of them had received a blood transfusion or plasma therapy at any stage, they were excluded from the serological analysis. This age group was selected because older animals generally had already left the farm area for various purposes. The serological status of the 6-24 months old horses therefore exclusively reflects the epidemiological conditions particular to each farm. Blood samples were centrifuged and the

serum from each sample was decanted into separate containers in the laboratory. The containers were then stored and maintained at -20°C until processed.

During visits to farms, tick infestations were evaluated on the horses, especially among the 6-24 month age group. Details of the presence and abundance of the ticks *D. nitens*, *A. cajennense*, and *R. (B.) microplus* infesting the horses of these 40 farms have been published elsewhere (LABRUNA et al., 2001). If any of these tick species were not found on the target age group (6-24 months old), older horses were also examined. The occurrence of each tick species was categorized qualitatively per farm as follows: level zero: no ticks on the horses; low-level infestation: mean of less than 10 adult ticks per horse; high-level infestation: mean of more than 10 adult ticks per horse (LABRUNA et al., 2001). Animals that had been recently treated with acaricides were not included in these assessments. Adult ticks were only considered for this analysis because farms were visited during the summer, when adults of *A. cajennense* are in their highest peak of abundance (LABRUNA et al., 2002). For *D. nitens* and *R. (B.) microplus*, both one-host ticks, adults are found throughout the year, but also with major peaks encompassing summer months (LABRUNA et al., 2002; CAMPOS PEREIRA et al., 2008).

On each farm visit a questionnaire was served to the farm supervisor with the purpose of gaining information about horse management, especially of the historical usage of discarded needles on the horses for any therapeutic purpose and the presence of horse fly (Tabanidae) infestations on the horses.

3. Serological testing

All serum samples were subjected to the CFT and a competition inhibition ELISA (cELISA) test for *B. caballi* and *T. equi*. The CFT test was performed following the protocol of the United States Department of Agriculture (USDA, 1997), which also supplied the reagents. Trace, 1+ to 4+ was considered as positive reactions in a 1:5 dilution. The cELISA test was performed as described elsewhere (KNOWLES et al., 1991; 1992; KAPPEMEYER et al., 1999). Both serologic tests were performed with USDA standardized antigens and reagents, which have been regularly supplied by USDA to the Laboratório Paddock de Análises Clínicas, São Paulo, Brazil, where serologic tests were performed for the present study, under C. E. Kerber.

4. Statistical analysis

The proportions of horses in the 6-month to 24-month age group that were serologically positive for *B. caballi* and *T. equi* were calculated separately for each farm, for each one of the serological assays (CFT and cELISA). For data analysis, a logistic regression was performed to construct multivariate models that could explain the dependent variable (horses serologically positive for *B. caballi* or *T. equi*) as a function of the independent variables (presence and abundance of each one of the tick species found on the farms). For this purpose, infestations of horses by *D. nitens*, *A. cajennense* or *R. (B.) microplus* were analyzed qualitatively at a dichotomous level (absence = 0; presence = 1) or at a semi-quantitatively

dichotomous level (level zero and low level of infestation = 0; high level of infestation = 1). The serological results of each farm were analyzed at two separate dichotomous levels: firstly, with the presence or absence of positive horses (no positive horse = 0; at least one positive horse on the farm = 1) and secondly, at the proportion of positive horses on the farm (less than 30% of horses positive = 0; more than 30% of horses positive = 1). A separate statistical analysis was performed for each serological test (CFT and cELISA).

The independent variables were subjected to univariate analysis and those with statistical association (here considered as $P < 0.20$, χ^2 test, for this first analysis) were tested in the multivariate model by 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. In the case of *T. equi*, the probability values of horses to be infected by this agent were calculated, considering the significant independent variables indicated by the multivariate analysis. A *Kappa* coefficient was calculated for the serological results shown by CFT and cELISA among all sera samples. All analyses were performed using SPSS for Windows (1999).

Results

1. Overall serologic results

A total of 582 sera samples were obtained from horses on the 40 farms (mean: 14.55 ± 11.98 horses per farm, range: 4-60) and tested by CFT and cELISA. The results of these tests are given in Table 1. There was an overall higher prevalence of *B. caballi* (54.1%) than of *T. equi* (21.6%) infected horses. There were discrepancies between the CFT and cELISA results, with the cELISA detecting more positive and less negative *B. caballi* horses than the CFT, while, quite unexpectedly, the opposite was true for *T. equi*. The *Kappa* coefficients between the two tests were 0.67 for *B. caballi* and 0.70 for *T. equi*, indicating a substantial agreement between the CFT and cELISA (THRUSFIELD, 1995).

Comparing the two serological tests, 31 and 32 farms had at least one positive horse for *B. caballi* by CFT and cELISA, respectively, and 24 and 23 farms had at least one positive horse for *T. equi* by CFT and cELISA, respectively (Table 2). Considering the level of infection on the farms, 26 and 31 farms showed more than 30% of horses positive for *B. caballi* by CFT and cELISA, respectively, and 11 and 12 farms showed more than 30% of horses positive for *T. equi* by CFT and cELISA, respectively.

2. Ticks

Three tick species were found on the horses during the study (Table 2). The most common species, *D. nitens*, was present on 38 farms (95%), with 22 farms (55%) showing high-level infestations. *Amblyomma cajennense* was present on 20 farms (50%), with 8 farms (20%) showing high infestations on horses. The third species, *R. (B.) microplus*, was present on horses from

Table 1. Comparison of serologic tests of 582 serum samples from 6 to 24-month-old horses from São Paulo, Brazil.

Test results	N° of reactive serum samples (% positive)	
	<i>Babesia caballi</i>	<i>Theileria equi</i>
Positive by CFT	318 (54.6)	166 (28.5)
Positive by cELISA	405 (69.6)	155 (26.6)
Positive by both tests	315 (54.1)	126 (21.6)
Negative by CFT	264 (45.4)	416 (71.5)
Negative by cELISA	177 (30.4)	427 (73.4)
Negative by both tests	174 (29.9)	387 (66.5)
Positive by CFT, negative by cELISA	3 (0.5)	40 (6.9)
Negative by CFT, positive by cELISA	90 (15.5)	29 (5.0)

N° = number.

Table 2. Serological tests for *Babesia caballi* and *Theileria equi* of horses from 40 stud farms, according to the tick species found on horses on the farms.

Tick species found on at least one horse on the farm ^a	N° of farms	N° of farms with at least one horse positive for <i>B. caballi</i> (%)		N° of farms with at least one horse positive for <i>T. equi</i> (%)		N° of farms with more than 30% of horses positive for <i>B. caballi</i> (%)		N° of farms with more than 30% of horses positive for <i>T. equi</i> (%)	
		CFT	cELISA	CFT	cELISA	CFT	cELISA	CFT	cELISA
A	15	14 (93.3)	15 (100)	9 (60)	7 (46.6)	11 (73.3)	14 (93.3)	1 (6.6)	2 (13.3)
A and B	19	13 (68.4)	13 (68.4)	12 (63.1)	13 (68.4)	11 (57.8)	13 (68.4)	8 (42.1)	8 (42.1)
A and C	3	3 (100)	3 (100)	2 (66.6)	2 (66.6)	3 (100)	3 (100)	1 (33.3)	1 (33.3)
A, B, and C	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
no ticks	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	40	31 (77.5)	32 (80)	24 (60)	23 (57.5)	26 (65)	31 (77.5)	11 (27.5)	12 (30)

^aA: *Dermacentor nitens*; B: *Amblyomma cajennense*; C: *Rhipicephalus (Boophilus) microplus*. N° = number.

only 4 farms (10%), of which 2 had high infestations. Only 2 farms were completely free of tick infestation on horses. All the farms that had *A. cajennense* or *R. (B.) microplus* infestations also had *D. nitens* infestations. Only 1 of the 4 farms with *R. (B.) microplus* infestations on horses also had *A. cajennense* infestations. Table 2 and 3 summarize the serological status of the farms in respect to the tick species found on the horses.

All farms reported using only sterile disposable needles for treatment of horses and always discarding needles after use. All farms also reported infestations by horse flies (presumably Tabanidae) on horses every year, with high fly burdens occurring during the spring-summer months.

3. *Babesia caballi*

Both the CFT and the cELISA results gave similar statistical associations when compared to the independent variables related to tick species found on horses on the farms (Table 4). By the univariate analysis, the presence of at least one serologically positive horse for *B. caballi* by CFT was statistically coupled ($P < 0.20$) with both the presence and the high-level infestation of *D. nitens* on the horses. However, when these two independent variables were subjected to the multivariate analysis, the presence of *D. nitens* was excluded and only its high level of infestation (mean infestation of more than 10 adult ticks per horse) was statistically associated with

the presence of at least one horse positive for *B. caballi* on CFT (model significance $P = 0.0001$). Identical significant associations were observed when the presence of at least one horse serologically positive for *B. caballi* by cELISA on the farm was compared to the independent variables (Table 4).

Similarly, the univariate analysis indicated that the presence of more than 30% of horses on the farm positive for *B. caballi*, by either CFT or cELISA, was also statistically coupled ($P < 0.20$) with the presence and with high-level infestation by *D. nitens* on the horses. Again, when these two independent variables were subjected to the multivariate analysis, only the high-level infestation by *D. nitens* was statistically associated with more than 30% of horses positive for *B. caballi* on the farm, by either CFT or cELISA. Both models (one for each serological test) were highly significant ($P = 0.0001$).

4. *Theileria equi*

By the univariate analysis, the presence of at least one horse serologically positive for *T. equi* by CFT was statistically coupled ($P < 0.20$) with three variables: the presence of *D. nitens*, high-level infestation by *D. nitens*, and high-level infestation by *A. cajennense* on the horses. The cELISA results indicated that the above three variables and a fourth, the presence of *A. cajennense* on the horses, were significant in the univariate analysis ($P < 0.20$). However, when

Table 3. Serological tests on horses from 25 stud farms which had high infestation levels by ticks on the horses.

Tick species found at high infestation level on the horses ^a	N° of farms	N° of farms with at least one horse positive for <i>B. caballi</i> (%)		N° of farms with at least one horse positive for <i>T. equi</i> (%)		N° of farms with more than 30% of horses positive for <i>B. caballi</i> (%)		N° of farms with more than 30% of horses positive for <i>T. equi</i> (%)	
		CFT	cELISA	CFT	cELISA	CFT	cELISA	CFT	cELISA
A	16	16 (100)	16 (100)	13 (81.2)	10 (62.5)	16 (100)	16 (100)	2 (12.5)	3 (18.7)
B	3	2 (66.6)	2 (66.6)	3 (100)	3 (100)	1 (33.3)	2 (66.6)	3 (100)	2 (66.6)
A and B	4	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
A and C	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
A, B, and C	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total	25	24 (96)	24 (96)	22 (88)	19 (76)	23 (92)	24 (96)	11 (44)	11 (44)

^aA: *Dermacentor nitens*; B: *Amblyomma cajennense*; C: *Rhipicephalus (Boophilus) microplus*. N° = number.

Table 4. Results of statistical analysis between the presence of tick species found on the horses and the horse serological status on the farm for *Babesia caballi* or *Theileria equi* by CFT or cELISA.

Tick species on the horses	P-values of univariate analysis for serologically positive horses by:							
	CFT				cELISA			
	At least one horse positive for <i>B. caballi</i>	At least one horse positive for <i>T. equi</i>	More than 30% of horses positive for <i>B. caballi</i>	More than 30% of horses positive for <i>T. equi</i>	At least one horse positive for <i>B. caballi</i>	At least one horse positive for <i>T. equi</i>	More than 30% of horses positive for <i>B. caballi</i>	More than 30% of horses positive for <i>T. equi</i>
Presence of <i>D. nitens</i>	0.121 ^a	0.057 ^a	0.037 ^a	0.548	0.096 ^a	0.069 ^a	0.009 ^a	0.541
Presence of <i>A. cajennense</i>	0.712	0.301	0.816	0.007 ^a	0.442	0.184 ^a	0.712	0.023 ^a
Presence of <i>R. (B.) microplus</i>	0.557	0.638	0.278	0.300	0.566	0.624	0.557	0.570
High level infestation by <i>D. nitens</i>	<0.001 ^{a, b}	<0.001 ^{a, b}	<0.001 ^{a, b}	0.286	0.001 ^{a, b}	0.031 ^{a, b}	<0.001 ^{a, b}	0.096 ^a
High level infestation by <i>A. cajennense</i>	0.655	0.013 ^{a, b}	0.689	<0.001 ^{a, b}	1.000	0.013 ^{a, b}	0.655	<0.001 ^{a, b}

^aSignificantly at the univariate analysis (P < 0.20). ^bIncluded in the final multivariate model (model significance P < 0.05).

all these independent variables were submitted to the multivariate analysis with either CFT or cELISA results, only the high level of infestation by *D. nitens* or *A. cajennense* were statistically associated with the presence of at least one horse positive for *T. equi* on the farm. Both models (one for each serological test) were highly significant (P < 0.001).

The univariate analysis indicated that the presence of more than 30% of horses positive for *T. equi* on the farm by CFT was statistically coupled (P < 0.20) with two variables: the presence of *A. cajennense*, and its high-level infestation on horses. In comparison, the cELISA results showed that with more than 30% of *T. equi*-positive horses on the farm, the above two variables and a third, high level of infestation by *D. nitens* on the horses, were significant in the univariate analysis (P < 0.20). However, when these independent variables were submitted to the multivariate analysis, only the high-level infestation by *A. cajennense* was statistically associated with more than 30% of horses positive for *T. equi* on the farm by either CFT or cELISA (both model significances were P = 0.0001). The presence of *R. (B.) microplus* on the horses on

the farms was not statistically associated (P > 0.20) with any of the serological results (Table 4).

Probability values for farms to have more than 30% of horses serologically positive for *T. equi* were calculated. Using the CFT data, the probability that a farm with low-level infestation by *A. cajennense* would have more than 30% of horses positive for *T. equi* was 9.4%. If the farm had a high level of infestation by *A. cajennense* on the horses, this probability increases to 99.9%. Similarly, using the cELISA data, the probability that a farm with low-level infestation by *A. cajennense* would have more than 30% of horses positive for *T. equi* was 15.6%, whereas with a high-level infestation by *A. cajennense* on the horses, this probability increased to 87.5%.

Discussion

The present study reinforces previous studies that showed horse piroplasmosis to be endemic in Brazil (BARBOSA et al., 1995; BITTENCOURT et al., 1997; KERBER et al., 1999;

RIBEIRO et al., 1999; XUAN et al., 2001; BOTTEON et al., 2002; BALDANI et al. 2007; HEIM et al. 2007). In addition, it highlights the epidemiological associations between tick infestations and serologically positive results for *B. caballi* and *T. equi* in horses. The significant association ($P < 0.05$) between the high-level infestation by *D. nitens* on the horses and the presence of at least one *B. caballi*-positive horse was expected, as well as the more than 30% of positive horses on the farm, since it is well known that *D. nitens* is the vector of *B. caballi* in the New World (ROBY; ANTHONY, 1963). Thus, such association can be considered a control of our methods, in order to validate our association analyses between *T. equi* and ticks. In this context, we found significant association ($P < 0.05$) of high-level infestation by *A. cajennense* on farms with more than 30% *T. equi*-positive horses. This finding was surprising, since one experiment showed that this tick was not a competent vector of *T. equi* (DENNIG, 1988). Our results showed that high-level infestation by both *A. cajennense* and *D. nitens* was statistically associated ($P < 0.05$) with the presence of at least one horse positive for *T. equi* on the farm, although such association for *D. nitens* was not supported by further statistical analyses. In the case of *A. cajennense*, a high level of infestation was statistically associated ($P < 0.05$) with more than 30% of horses positive for *T. equi* on the farms (Table 4).

Guimarães et al. (1998) showed, under experimental conditions, that *R. (B.) microplus* was susceptible to infection by *T. equi* and was able to transmit it to horses. This finding was later confirmed by other workers (STILLER et al., 2002; UETI et al., 2005; 2008). Cattle are the primary host for *R. (B.) microplus* and it is well known that infestation with this tick on other host species (e.g., horses) is dependent on the presence of cattle in the same area (LABRUNA et al., 2001). In fact, horse infestation by *R. (B.) microplus* has always been reported from an area where cattle have been grazing (BITTENCOURT et al., 1990; HEUCHERT et al., 1999; LABRUNA et al., 2001). During the present study, information about the presence of cattle and their contact with horses was obtained and reported by Labruna et al. (2001), who found at least nine farms on which there were no cattle, either in the farm area or in adjacent areas, nor any *R. (B.) microplus* on the horses. However, at least one horse positive for *T. equi* by CFT occurred on each of these farms (data not shown). Moreover, two of these farms had more than 30% of the horses positive for *T. equi* by CFT. Similar conditions were found on seven farms, using the cELISA results. They did not have any cattle or *R. (B.) microplus* on the horses but had at least one horse positive for *T. equi* by cELISA. On one of these farms more than 30% of the horses were positive for *T. equi* (data not shown). Even though *R. (B.) microplus* could transmit *T. equi* under natural conditions in Brazil, our findings suggest that other transmission possibilities might exist. The high statistical association ($P < 0.05$) between the abundance of *A. cajennense* on horses and the presence of more than 30% of horses positive for *T. equi* suggests that *A. cajennense* could be acting as a vector of *T. equi* under natural conditions. This epidemiological indication is reinforced by the fact that all serum samples examined were from young horses (6-24 months old) that had never left the farm area for any reason, and had never received blood transfusions or plasma therapy at any stage of their lives. In addition, all farms having no

cattle reported the complete absence of any cattle on the farm for at least 36 months (data not shown), which was a larger period than the maximal age (24 months) of the tested horses. Thus, we can also state that there was no *R. (B.) microplus* established on the farm since the 6-24 month age group was born.

We are aware of the fact that the serologically positive horses detected in the present study became infected before the moment at which we collected their ticks and serum samples. However, we assumed that farms with *A. cajennense* infesting horses had this tick already established in the farm for at least one year because this tick completes only one generation per year in the state of São Paulo (LABRUNA et al., 2002). This statement is especially valid for those farms where we detected high infestation levels of *A. cajennense* on horses.

Excluding ticks as vectors, other possibilities for these horses to become infected with *T. equi* could include mechanical transmission or congenital infection. Mechanical transmission was minimized by the fact that all farms reported using only sterile disposable needles for any kind of therapy on the horses. Mechanical transmission of *T. equi* by biting flies has not been previously reported (FRIEDHOFF, 1988) and in this study, high infestations of horse flies during the summer-spring months were reported on all farms, even those where all the young horses were serologically negative for *T. equi*.

Intra-uterine infection of foals with *T. equi* has been reported. Some studies report that these foals are usually stillborn, showing the lesions characteristic of equine piroplasmiasis, or that infected foals born alive show clinical signs of the disease, which is invariably fatal (ERBSLÖH, 1975; MAIA et al., 2006). Other studies have shown that fetuses may become infected with *T. equi* without developing disease and are born as carriers of the parasite (LEWIS, 1998; ALLSOPP et al., 2007). While it has been argued that transplacental transmission of *T. equi* within a herd seems to be much more significant than previously thought (ALLSOPP et al., 2007), this is still a controversial issue (KUMAR et al., 2008). Nonetheless, transplacental transmission has not affected our results because it is very unlikely that this kind of transmission would coincidentally occur more frequently in farms with high-level infestation by *A. cajennense*, and where more than 30% of the horses were infected by *T. equi*.

To date, *R. (B.) microplus* is the only tick species of the Brazilian fauna proven experimentally to transmit *T. equi* to horses; however, its role in the epidemiology of equine piroplasmiasis should be evaluated under field conditions. Since *R. (B.) microplus* is a one-host tick and there is no transovarial transmission of *T. equi* in ticks (UETI et al., 2008), the only possible transmission of *T. equi* by this tick is by accidental transference of recently molted ticks or by males wandering from horse to horse. Mason and Norval (1981) showed that 69 feeding larvae and two adult males of *R. (B.) microplus* were able to transfer from one bovine (initially infested with 30,000 unfed larvae) to another under a high stocking rate of four cattle per 0.7 ha. Further studies should evaluate transfer rates of *R. (B.) microplus* ticks among horses under field conditions, which would have important implications in the transmission of *T. equi* in horses grazing on cattle pastures. Our epidemiological findings showing a significant association between *T. equi*-positive horses and infestations by *A. cajennense*, even on

farms where there were no *R. (B.) microplus* and cattle, suggest that other transmission mechanisms exist. Our epidemiological data indicates that *A. cajennense* is associated with *T. equi* infection in horses, in opposition to experimental studies that fail to prove *A. cajennense* as a competent vector. In fact, after our field study, we tried to infect *A. cajennense* ticks by allowing nymphs to feed on a mare with high *T. equi* parasitemia ($\approx 70\%$), but no tick remained infected after molting to the adult stage (M.B. Labruna, C. Kerber, and M.F.B. Ribeiro, unpublished data). While further studies re-evaluating the biological transmission of Brazilian *T. equi* strains by *A. cajennense* are still needed, it is possible that other transmission mechanisms yet to be elucidated have their existence on the farm in coincidence with high levels of infestation by *A. cajennense*, which would explain part of our epidemiological associations. Although this remains unclear, our results indicate that prevention measures of *T. equi* infection in Brazil should be based on the control or elimination of *R. (B.) microplus* and *A. cajennense* infestations on horses. For *R. (B.) microplus*, this can be easily achieved by isolating from cattle. Infestation by *A. cajennense* can be controlled by keeping the pastures uniform and in a clean condition through mowing the entire horse pasture area (and removing the mowed grass) at least once a year, during the spring-summer months (LABRUNA et al., 2001). Successful strategic chemical control has also been achieved (LABRUNA et al. 2004).

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