



Control of equine piroplasmosis in Brazil

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ABSTRACT

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The importance of equine piroplasmosis control in endemic countries has increased in recent years and plays an important role to maintain the international market open to the horse industry. The purpose of this study was to demonstrate the occurrence of equine piroplasmosis (*Babesia equi* or *Theileria equi* and *Babesia caballi*) in Brazil—a country where the disease occurs endemically—in different climatic conditions, and to evaluate the results of a strategy for tick control in order to decrease infection rates. Blood samples were taken from 720 horses on 28 farms from different regions and subjected for complement fixation testing. The strategy was based on the control of the tick population by spraying the horses with acaricides, treating positive horses and preventing cattle and horses from grazing together. A significant association was found in the prevalence of antibody titres in tropical and subtropical areas. A significantly lower prevalence rate occurred on those farms where measures to control tick population were established. Farms in endemic countries may significantly reduce the prevalence of equine piroplasmosis by establishing measures to control the tick population and treating chronic carrier horses. Additional measures for controlling ticks in tropical areas are also discussed.

Keywords: *Babesia caballi*, *Babesia equi*, *Boophilus microplus*, equine piroplasmosis, tick, *Theileria equi*

INTRODUCTION

Tick-borne protozoan parasites of the phylum Apicomplexa cause disease in domestic animals, wild vertebrates and, in some instances, even man (Gorenflot, Moubri, Precigout, Carcy & Schetters 1998; Sonenshine 1993). The genera *Babesia* and *Thei-*

leria comprise very successful blood-infecting sporozoans of the subclass Piroplasmia (Levine, Corliss, Ox, Deroux, Grain, Honigberg, Leedale, Loeblich, Lom, Lynn, Merinfeld, Page, Poljansky, Sprage, Vavra & Wallace 1980) that have been recorded from most mammalian orders (Young & Morzaria 1986).

Among the Equidae, *Babesia equi*, recently reclassified by Melhorn & Schein (1998) as *Theileria equi*, and *Babesia caballi* are common and important intra-erythrocytic parasites and can be found in most tropical and subtropical areas of the world as well as in some temperate climatic zones (De Waal 1992).

According to Stiller & Coan (1995), very little information is available on the natural and/or experimental tick vectors of *B. equi* and *B. caballi* in the Western Hemisphere. To date, only the tropical horse tick, *Dermacentor nitens*, is known to be a natural vector for *B. caballi* in the New World (Roby & Anthony 1963). Guimarães, Lima & Ribeiro (1998) demonstrated that

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B. equi is capable of complete development in *Boophilus microplus* which, in turn, is capable of transmitting transstadially the parasite from one host to another, suggesting that this tick may function as a natural vector of *B. equi*.

In Brazil, *Amblyomma cajennense* and *D. nitens* are the commonest and most widespread ticks of horses. Although *B. microplus* principally infests cattle, it often occurs on horses kept with infested cattle (Stiller & Coan 1995).

The complement fixation test (CFT) was one of the earliest serological tests used for diagnosis of equine

piroplasmosis. It is still the prescribed serologic test to detect possible carriers (Holbrook, Frerichs & Allen 1972; Barriga 1997) and to regulate the entrance of horses into most countries in the world (Friedhoff, Tenter & Muller 1990).

Despite the severity of the acute phase of this disease in endemic areas such as Brazil, most recovered animals become carriers (De Waal, Van Heerden, Van den Berg, Stegman & Potgieter 1988). These animals remain in this state for long periods during which they act as a source of infection to vector ticks (Phipps 1996).



FIG. 1 Distribution of samples in tropical (MS, SP, MG) and subtropical (PR, RS) states of Brazil

Economic implications associated with equine piroplasmiasis include the cost of treatment, especially in acutely infected horses, abortions, loss of performance, death (Guimarães, Lima, Tafuri, Ribeiro & Sciacavico 1997) and restrictions in meeting international requirements concerning exportation or participation in equestrian sports (Van Heerden 1996). In recent years, the international movement of horses has increased the significance of the disease due to the risk of its transmission from carrier horses to a naive population. In endemic countries, the control of equine piroplasmiasis plays an important role to maintain the international market open to the horse industry (Friedhoff 1988).

Considering that the carrier state continues for years and that there are limitations in the sterilization of *B. equi* carriers, a logical approach would be to control the disease to prevent horses from becoming infected. As ticks are the most significant vectors, their control forms an important aspect in piroplasmiasis transmission.

In Brazil (Fig. 1), the states of Mato Grosso (MS), Sao Paulo (SP), Parana (PR), Rio Grande do Sul (RS) and Minas Gerais (MG) are the main localities of the horse industry. PR and RS, situated in the south of Brazil, have a subtropical climate, with average temperatures ranging between 14 and 35 °C in summer and between 0 and 20 °C in winter. Temperatures can drop below zero in these areas in the winter. In the states of MT, MG and SP, the predominant climate is tropical, with temperatures ranging between 17 and 35 °C in summer and 10 and 25 °C in winter. Temperatures around zero are uncommon.

The purpose of the current study was to demonstrate the occurrence of equine piroplasmiasis (*B. equi* and *B. caballi*) infections, under different climatic conditions, in a country in which it occurs endemically, and to evaluate the results of a strategy for tick control designed to decrease the infection rate.

MATERIAL AND METHODS

Blood samples from 720 horses were collected in siliconized vacutainer tubes and were allowed to clot. The samples were centrifuged and the serum from each sample was decanted into separate containers. The containers were stored and maintained at -20 °C until processing. The horses were from 28 farms located in different regions in Brazil (Table 1). On 18 of the farms Thoroughbred were bred (TB), on three Arabians (AR), on one Andalusians (AN) and on six Mangalarga (ML—a Brazilian breed). Random samples were taken.

The CFT for equine piroplasmiasis detection was performed following the protocol of the United States Department of Agriculture (Anon 1992), which also

supplied the reagents. Trace reactions were considered negative while 1+ to 4+ was considered positive in a 1:5 dilution.

In controlled farms, we recommended spraying the entire horse's body at least every three weeks and surrounding areas from time to time with acaricide in order to destroy ticks. A regime of spraying Deltamethrine (Butox, Hoechst Brasil), at a dilution of 25 mg/l every 3 weeks for at least 1 year in tropical areas was recommended, aimed at reducing infestation on the pasture. In subtropical areas we considered every three weeks in the spring and summer seasons to be sufficient to control tick population. Considering the possibility of *B. microplus* as a potential vector (Guimaraes, Lima & Ribeiro 1998), the removal of cattle from the same paddock was recommended. If possible, they entered in the control program. After at least one year adopting this regime, farms were considered controlled.

Some uncontrolled farms were attempting a partial tick control, spraying acaricide or sporadically using a paste in the horses' ears. On some other uncontrolled farms, treatment with babesicidal drugs had recently been introduced. These isolated measures that did not prevent new infections were considered as ineffective and the farms as uncontrolled.

The treatment with babesicidal drugs on controlled farms was recommended if there was any clinical sign of the disease and also in positive cases (chronic carriers) to aid in decreasing antibody titres. Imidocarb dipropionate (Imizol, Coopers Brasil Ltda.), at doses and intervals recommended by the manufacturer for *B. equi* infections (2 x 4,4 mg/kg/24 h) was used.

Results were analyzed by logistic regression analysis (Hosmer & Lemeshow 1989) comparing the prevalence of *B. caballi* and *B. equi* on farms with and without tick control and also in regions of tropical and subtropical climates.

RESULTS

A significant association ($P < 0,0001$) in the prevalence of antibody titres in tropical and subtropical areas and on farms with and without tick control was evident (Table 2).

The relationship between the dependent variable, seropositivity for *B. equi* and *B. caballi*, and the independent variables, climate and tick control, were assessed. Lack of tick control was found to be a risk factor for *B. equi* seropositivity (OR = 10,6). Risk factors for *B. caballi* seropositivity were lack of tick control (OR = 9,6) and tropical climate (OR = 4,1).

Substituting the risk factors in a multivariate statistical model obtained by logistic regression, the probabilities of an animal to be seropositive for *B. equi* in

TABLE 1 Distribution of samples by farm, state and breed and number of positive samples for *B. equi* and *B. caballi* from tropical and subtropical areas ($n = 720$)

Farm	State	Breed	Positive <i>B. caballi</i>	Positive <i>B. equi</i>	Total samples
Subtropical areas					
6	RS	TB	0	5	7
7	RS	TB	0	5	76
10	RS	TB	5	8	8
13	RS	TB	0	9	32
18	PR	TB	0	5	5
20	RS	TB	6	13	101
23	PR	TB	1	0	12
24	PR	TB	0	3	9
26	PR	TB	0	0	11
Total			12	48	261
Tropical areas					
1	MS	TB	15	14	31
2	MG	ML	27	66	95
3	MG	AR	5	10	10
4	MG	ML	0	14	16
5	MG	ML	3	9	9
8	SP	ML	10	10	17
9	SP	AR	8	7	9
11	SP	TB	6	0	10
12	SP	TB	6	0	89
14	SP	AN	4	5	8
15	SP	TB	13	0	15
16	SP	ML	1	3	15
17	SP	TB	31	9	42
19	MG	AR	0	6	6
21	SP	ML	8	25	29
22	SP	TB	11	5	15
25	SP	TB	0	0	7
27	SP	TB	0	6	22
28	SP	TB	7	6	14
Total			155	195	459

AN = Andalusian
 AR = Arabian
 MG = Minas Gerais
 ML = Mangalarga
 MS = Mato Grosso
 PR = Paraná
 RS = Rio Grande do Sul
 SP = São Paul
 TB = Thoroughbred

TABLE 2 Comparison between the prevalence of *B. caballi* and *B. equi* antibody titres in farms with and without tick control and in tropical and subtropical areas

	Controlled (%)	Uncontrolled (%)
<i>B. caballi</i>	3,75	38,75
<i>B. equi</i>	10,31	52,20
	Tropical (%)	Subtropical (%)
<i>B. caballi</i>	33,77	4,60
<i>B. equi</i>	42,48	18,39

uncontrolled farms was 52,5% and for *B. caballi* in tropical and subtropical areas were 42,8% and 15,5%, respectively. Tick control reduced these probabilities to 9,4% for *B. equi* and to 7,3% and 1,9%, for *B. caballi* in tropical and subtropical areas, respectively.

There was no success in eliminating infection for equine piroplasmosis in any of the controlled farms.

DISCUSSION

A substantial difference in the prevalence of titres between controlled and not controlled farms was recorded. However, complete elimination of titres was not achieved with the measures established on controlled farms. Even when the *B. equi* infection was treated with babesicidal drugs, in most cases we observed that antibody titres persisted over a long period (Tenter & Friedhoff 1986). Probably the treatment was not effective to sterilize the horse (Singh, Banerjee & Gautam 1980, Kuttler, Zaugg & Gipson 1987). In cases of *B. caballi* infections the treatment usually is effective in eliminating the parasite (Frerichs, Holbrook & Johnson 1969; Frerichs & Holbrook 1974). Another point to be considered is that the horses on the controlled farms were not isolated. Mares frequently travelled to other farms, increasing the possibility of new infections. Finally, the strategy of just spraying the horses' bodies and facilities with acaricide was not enough to eliminate the entire population of ticks on the controlled farms.

Some farms from the non controlled group showed a very low prevalence of antibody titres (numbers 23, 25 and 26). On these farms, as a result of the occupation, the rate was also very low.

Regarding the difference between *B. caballi* and *B. equi* occurrence, in tropical and subtropical areas, we could demonstrate that climate affects the occurrence of *B. caballi* and *B. equi* causing a lower prevalence in subtropical areas. Probably this is also related to the optimal development of the tick population, but this was not evaluated since very little information is available on the natural and/or experimental tick vectors of *B. caballi* and *B. equi* in the Western Hemisphere (Stiller & Coan 1995).

On farms where cattle shared the same field with horses (2, 3, 4, 5, 10 and 18) and where there was no tick control, some of the highest prevalence in this study, especially for *B. equi* infections, was found.

As demonstrated, it is possible to achieve a substantial lower number of positive horses just by controlling the tick population. This can be done with artificial measures to control the tick population, but it also happens naturally in lower temperature and humidity areas. The results of our strategy may be improved

with additional measures such as rotational use of the pasture which may be better evaluated in further studies.

In enzootic areas, constant infections induce immunity and endemic stability. Hence, clinical diseases are rather seldom observed in these regions (Schein 1988). Breakdown of the immune defense and a new recrudescence of the infection only occur under extremely stressful situations (Taylor, Bryant, Anderson & Willers 1969). Control of infection is desirable in order to avoid abortions, intrauterine transmission, clinical disease in stressful situations and constraints in exportation and participation in equestrian sports.

Controlling the tick population is an easy way to control infections and to improve the quality of horse populations in endemic areas. It is probable that chronic carriers, negative at CFT, may in the near future be detected by new and more sensitive tests, such as ELISA, PCR or Immunoblot.

These tests may be established by non-endemic countries to control the entrance of horses and, in this case, the sanitary barriers will become a serious limitation for the horse industry in endemic countries.

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