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Factors associated to *Theileria equi* in equids of two microregions from Rio de Janeiro, Brazil

Fatores associados à *Theileria equi* em equídeos de duas microrregiões do Rio de Janeiro, Brasil

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Abstract

Serum samples from 714 equids of Itaguaí and Serrana microregions, Rio de Janeiro, southeastern Brazil, were examined by indirect fluorescent antibody test (titer 1:80) for *Theileria equi*. The prevalence in the microregions and factors associated with seropositivity were evaluated and the prevalence ratio (PR) calculated. The overall prevalence of *T. equi* infection was 81.09% (n = 579), with higher prevalence ($p < 0.05$) in the Itaguaí (85.43%) when compared to Serrana microregion (76.92%). The geographic area, altitude, farming condition and area of origin of equids were associated ($p < 0.05$) with seropositivity for *T. equi*. Equids reared in the Itaguaí microregion (PR = 1.11, p = 0.003) and at altitudes below 500 m (PR = 1.10; p = 0,014) were more likely to be seropositive for *T. equi*. Furthermore, when equids were born in the farm (PR = 1.10, p = 0.008) and reared with poor farming conditions (PR = 1.13, p = 0.018) they were more likely to be exposed to *T. equi*. The main ticks found on equids were *Amblyomma cajennense* and *Dermacentor (Anocentor) nitens*. The microregions studied are endemic areas for equine theileriosis and there exists enzootic stability for *T. equi*. Only factors related to the collection area of serum samples influenced the seropositivity of equids for *T. equi* in that region.

Keywords: horses, equine theileriosis, IFAT, prevalence, epidemiology.

Resumo

Amostras de soro de 714 equídeos das microrregiões de Itaguaí e Serrana, Rio de Janeiro, Brasil, foram submetidas ao teste de imunofluorescência indireta (título 1:80) para *Theileria equi*. A prevalência entre as microrregiões e os fatores associados à soropositividade foram avaliados e a razão de prevalência (RP) calculada. A prevalência geral para *T. equi* foi de 81,09% (n = 579), com maior prevalência ($p < 0,05$) para microrregião de Itaguaí (85,43%), quando comparado a Serrana (76,92%). A região, altitude, nível da propriedade e origem dos equídeos foram associados ($p < 0,05$) com a soropositividade para *T. equi*. Equídeos criados na microrregião de Itaguaí (RP = 1,11; p = 0,003) e em altitudes abaixo de 500 m (RP = 1.10; p = 0,014) apresentaram maior chance de se tornarem soropositivos para *T. equi*. Além disso, quando são criados em propriedades de nível ruim (RP = 1,13; p = 0,018) e nascidos na propriedade (RP = 1,10; p = 0,008) apresentaram mais chance de terem contato com *T. equi*. As principais espécies de carrapatos encontradas parasitando os equídeos foram *Amblyomma cajennense* e *Dermacentor* (*Anocentor*) *nitens*. As microrregiões estudadas são endêmicas para theileriose equina e de estabilidade enzoótica para *T. equi*. Apenas os fatores relacionados à área de coleta das amostras de soro influenciaram a soropositividade dos equídeos para *T. equi* naquela região.

Palavras-chave: equinos, theileriose equina, RIFI, prevalência, epidemiologia.

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Introduction

Equine theileriosis, also known as equine piroplasmosis, is a disease caused by *Theileria equi*, an intraerythrocytic parasite that infects equids. This agent is biologically transmitted by ixodid ticks (THOMPSON, 1969). In Brazil, *T. equi* is transmitted transstadially and intrastadially by *Rhipicephalus (Boophilus) microplus* (GUIMARÃES et al., 1998; UETI et al., 2005, 2008). *Theileria equi* can also be transmitted iatrogenically through infected blood (TENTER; FRIEDHOFF, 1986), transplacentally (ALLSOPP et al., 2007) and congenitally (SANTOS et al., 2008). Clinical signs of the disease in equids are fever, intravascular hemolysis, anemia, icterus, hemoglobinuria, edema, depression and even death (DE WALL, 1992). The severity of clinical signs is variable, and in many cases spontaneous recovery may occur without manifest clinical signs, especially in endemic areas (RIBEIRO et al., 1995).

Equine theileriosis has a worldwide distribution and is endemic in most tropical and subtropical areas as well as in some temperate regions (DE WAAL, 1992). This distribution is closely related to areas of higher concentration of vector ticks (FRIEDHOFF, 1988). In Brazil, equine theileriosis is endemic and the identification of risk factors associated with *T. equi* infection may play a role in the adoption of control measures. Moreover, epidemiological evidence may help better understand the mechanisms by which *T. equi* is spread in the host population.

Studies have reported the association of tick infestation (KERBER et al., 2009), age (RIBEIRO et al., 1999; RÜEGG et al., 2007; KOUAM et al., 2010), breed system BOTTEON et al., 2002; MORETTI et al., 2010), horse farm activity, geographic area, season of the year and geldings (SHKAP et al., 1998; RÜEGG et al., 2007; KOUAM et al., 2010; MORETTI et al., 2010) with *T. equi* infection. However, other factors may be associated to infection because the epidemiological characteristics of disease may differ between regions.

The present study aimed to determine the prevalence of *T. equi* in equids and whether there exists enzootic stability for *T. equi* in the microregions studied and to identify factors associated with seropositivity in equids.

Materials and Methods

The study was carried out in the Itaguaí and Serrana microregions in the State of Rio de Janeiro, southeastern Brazil, between January and May 2009. Non-probability convenience sampling was used. Overall, 714 equids (702 horses and 12 mules), 350 from the Itaguaí microregion (Itaguaí, n = 116; Mangaratiba, n = 90; and Seropédica n = 144) and 364 from the Serrana microregion (Petrópolis, n = 192; and Teresópolis n = 172) were examined and blood and ticks were collected. The altitude was recorded at each collection site with the use of Global Positioning System (GPS). An epidemiological questionnaire was applied to identify factors associated with seropositivity to *T. equi* (animal species, collection area, gender, age, altitute, equids farm activity, area of origin of equids, parasitism by ticks and flies).

Blood samples were collected by jugular venipuncture using a vacuum system and placed in sterile tubes without anticoagulant. Serum samples were subsequently obtained by centrifugation at 6000 x g for 10 minutes and stored at –20 °C until serological analysis by indirect fluorescent antibody test (IFAT).

The horses and mules were inspected visually in the ears, head, neck, pectoral, armpit, inguinal and tail areas for identification of animals infested with ticks. In parasitized animals, the degree of infestation by each tick species was categorized according to Teglas et al. (2005) as: none, mild (1-20 ticks per animal), moderate (21-50 ticks per animals) or severe (more than 50 ticks per animal). Adult ticks were collected and stored in isopropyl alcohol for identification using an appropriate dichotomous key (BARROS-BATTESTI et al., 2006).

IFAT was performed using slides with partially purified antigen from Jaboticabal *T. equi* strain (GenBank accession nr. AF255730) previously prepared according to Baldani et al. (2007). For testing, the slides were incubated with each test serum, diluted at 1:80 in a humid chamber at 37 °C for 45 minutes. After three washes in buffered saline (PBS), the slides were incubated with fluorescein isothiocyanate-conjugated equine anti-immunoglobulin G (Sigma-Aldrich®) diluted at 1:32 and examined under a fluorescence microscope (Hund Wetzlar, model H600/12, Germany). Serum samples with titers ≥80 were considered positive. The positive control used was horse serum experimentally infected with Jaboticabal *T. equi* strain. The negative control used was horse serum free of infection by *T. equi* and *B. caballi*.

For statistical analysis, the following factors were analyzed: collection area (microregions and their municipalities); altitude (< 500 m; ≥500 m but < 800; and ≥800 to 1004 m); animal species (horse; mule); gender; age (< 6 months; 6-12 months; 1-3 years; 3-5 years; 5-10 years; and >10 years); equids farm activity (sports, exhibition, recreation, farming, breeding and foals of undetermined activity); breeding systems (confined, semi-confined and extensive); close contact with cattle (yes; no); and area of origin of equids (inside or outside the farm). In addition to these factors, the farms were categorized into four levels based on farming conditions (i.e., farm infrastructure and management of animals) as follows: excellent (animals reared in stalls and paddocks, fed with forage and concentrate rations, systematic control of ticks, no tick infestation, frequent veterinary care); good (same as above, except for non-systematic tick control and tick infestation); moderate (animals reared in stalls and pasture, sporadic tick control, tick infestation, sporadic veterinary care) and poor (animals reared on pasture only, no tick control, tick infestation and no veterinary care).

The prevalence of *T. equi* infection in the microregions and the frequency of positive equids in other categories were calculated, and potential differences were assessed using the chi-square and Fisher's exact test at 5% significance level. BioStat 4.0 was used to estimate the prevalence ratio (PR) for factors associated ($p < 0.05$) with seropositivity to *T. equi*.

Results

The overall *T. equi* prevalence was 81.09% (n = 579) in the equids examined (Table 1). The Itaguaí microregion showed a higher prevalence ($p < 0.05$) than that in the Serrana microregion,

Table 1. Prevalence of anti-*T. equi* antibodies in equids in the Itaguaí and Serrana microregions in the State of Rio de Janeiro and factors associated to the collection area.

Factor	N	*Theileria equi* n	*Theileria equi* %	p value
Prevalence	714	579	81.09	
Microregion				
Serrana	364	280	76.92[b]	0.005
Itaguaí	350	299	85.43[a]	
Municipality				
Itaguaí	116	104	89.66[a]	
Seropédica	144	123	85.42[a]	
Mangaratiba	90	72	80.00[a]	0.021
Teresópolis	172	133	77.33[b]	
Petrópolis	192	147	76.55[b]	
Altitude (m)				
<500	352	300	85.23[a]	
500 to <800	163	125	76.69[b]	0.021
800 to <1004	199	154	77.39[b]	
Farming conditions				
Excellent	127	97	76.38[b]	
Good	270	210	77.78[b]	0.038
Moderate	134	114	85.07[ab]	
Poor	183	158	86.34[a]	
Area of origin of equids				
Inside the farm	218	189	86.70[a]	0.015
Outside the farm	496	390	78.63[b]	

[a]Values followed by the same letters did not show any differences in the chi-square test at 5% significance level; N: number of animals examined by indirect fluorescent antibody test; n: number of seropositive animals.

Table 2. Prevalence of anti-*T. equi* antibodies in equids in the Itaguaí and Serrana microregions in the State of Rio de Janeiro and equid-related factors.

Factor	N	*Theileria equi* n	*Theileria equi* %	p value
Prevalence	714	579	81.09	
Animal species				
Horse	702	569	81.05[a]	0.432
Mule	12	10	83.30[a]	
Gender				
Male	416	334	80.29[a]	0.581
Female	298	245	82.21[a]	
Age				
<6 months old	30	27	90.00[a]	
6-12 months old	32	28	87.50[a]	
1-3 years old	85	70	82.35[a]	0.166
3-5 years old	92	73	79.35[a]	
5-10 years old	276	211	76.45[a]	
>10 years old	199	170	85.43[a]	
Equids farm activity				
Sports	279	229	82.08[a]	
Exhibition	47	40	85.11[a]	
Recreation	146	111	76.03[a]	0.375
Farming	111	87	78.38[a]	
Breeding	108	92	85.19[a]	
Foals of undetermined activity	23	20	86.96[a]	
Breeding system				
Confined	52	38	73.08[a]	
Semi-confined	498	405	81.33[a]	0.279
Extensive	164	136	82.93[a]	
Close contact with cattle				
No	521	427	81.96[a]	0.388
Yes	193	152	78.76[a]	

[a]Values followed by the same letters did not show any differences in the chi-square test at 5% significance level; N: number of animals examined by indirect fluorescent antibody test; n: number of seropositive animals.

85.43% (n = 299) vs. 76.92% (n = 280) respectively. The microregions with their municipalities, altitude and farming conditions were associated ($p < 0.05$) with the rate of *T. equi* antibodies (Table 1). Equids reared in the Itaguaí microregion and its municipalities (PR = 1.11, p = 0.003) and at altitudes below 500 m (PR = 1.10; p = 0,014) were more likely to be seropositive for *T. equi*. Furthermore, when equids were born in the farm (PR = 1.10, p = 0.008) and reared in poor farming conditions (PR = 1.13, p = 0.018) they were more likely to be exposed to *T. equi* (Table 4).

Equid-related factors studied are presented in Table 2. There was no association (p = 0.544) between positive serology and animal species, with 81.1% (n = 569) and 83.3% (n = 10) of positivity found in horses and mules, respectively. There was no association between gender (p = 0.581), age (p = 0.166), horse farm activity (p = 0.375), breeding system (p = 0.279) and close contact between equids and cattle (p = 0.388) and *T. equi* seropositivity. However, there was not found significantly higher prevalence of *T. equi* infection in equids used for exhibition (85.1%, n = 40), breeding (85.19%, n = 92), sports (82.08%, n = 229) and foals with undetermined activity (86.96%, n = 20) compared to those used for recreation and farming. There was found a trend for higher seropositivity in equids reared in semi-confined and extensive systems when compared to those bred in confined systems, with a prevalence of 81.33% (n = 405), 82.93% (n = 136) and 73.08% (n = 38), respectively.

Ticks collected parasitizing equids were identified as *Amblyomma cajennense*, *Dermacentor* (*Anocentor*) *nitens* and *Rhipicephalus* (*Boophilus*) *microplus* species. The latter species was found only in two horses and was not included in the analysis (Table 3). Parasitism by at least one of these species was observed in 82.54% (n = 279) of the animals, however, no association (p = 0.399) with seropositivity was seen. Similarly, no association with the degree of infestation by *A. cajennense* and *D. nitens* with *T. equi* seropositivity was found. In both cases, there was no significantly higher rates of positive animals associated with increasing degrees of tick infestation. Moderate to severe infestation by *D. nitens* and *A. cajennense* tick was observed, respectively, in 17.09% (n = 122) and 14.16% (n = 101) of the animals examined. The parasitism of equids by *A. cajennense* and *D. nitens* was more severe in the Itaguaí compared with the Serrana microregion. However, *A. cajennense* tick was widely distributed in both microregions

Table 3. Prevalence of anti-*T. equi* antibodies in equids in the Itaguaí and Serrana microregions in the State of Rio de Janeiro and factors related to tick parasitism and *Stomoxys calcitrans* fly infestation.

Factor	N	*Theileria equi*		p value
		n	%	
Tick infestation				
No	376	300	79.79[a]	0.399
Yes	338	279	82.54[a]	
Degree of infestation (*Amblyomma cajennense*)				
None	376	300	79.79[a]	0.576
Mild	220	178	80.91[a]	
Moderate	76	65	85.53[a]	
Severe	42	36	85.71[a]	
Degree of infestation (*Dermacentor nitens)*				
None	487	391	80.29[a]	0.536
Mild	78	66	84.62[a]	
Moderate	97	82	84.54[a]	
Severe	52	40	76.92[a]	
***Stomoxys calcitrans* infestation**				
No	425	343	80.71[a]	0.824
Yes	289	236	81.67[a]	

[a]Values followed by the same letters did not show any differences in the chi-square test at 5% significance level; N: number of animals examined by indirect fluorescent antibody test; n: number of seropositive animals.

(data not shown). In addition to tick parasitism, fly infestation by *Stomoxys calcitrans* fly was seen in 81.67% (n = 236) of horses. However, no association (p = 0.824) was found between fly infestation and *T. equi* seropositivity.

Discussion

The prevalence of *T. equi* infection found in this study is corroborated by previous studies showing that equine theileriosis is endemic in the municipalities of the metropolitan mesoregion of Rio de Janeiro (PFEIFER BARBOSA et al., 1995; BITTENCOURT; MASSARD, 1997; BOTTEON et al., 2002; SANTOS et al., 2009). In these studies the prevalence of *T. equi* infection ranged from 73.6 to 100%, which can be explained by differences in the number of equids examined and serological test used for diagnosis. Few studies have been conducted on equine theileriosis in the Serrana microregion of the state of Rio de Janeiro. Seropositivity for *T. equi* was seen in 21% (n = 52) of thoroughbred horses in an equestrian establishment in Teresópolis (DA COSTA PEREIRA et al., 2005) and 5.6% (n = 4) in Nova Friburgo (DA COSTA PEREIRA et al., 2007). The seroprevalence found in these studies is lower than that found in the Serrana microregion, 76.92% (n = 280) (Table 1). This inconsistency can be explained by a lower number of equids investigated in these studies, no distribution of sampling in the mountainous region and use of the complement fixation test (CFT) as a diagnostic method. According to Tenter and Friedhoff (1986) and Brüning (1996), the CFT is less sensitive than IFAT

Table 4. Prevalence ratio (PR) of anti-*Theileria equi* antibodies by IFAT* and factors associated with seropositivity in equids.

Factor	N	*Theileria equi*		PR	95% CI	p-value
		n	%			
Microregion						
Serrana	364	280	76.92	1		0.003
Itaguaí	350	299	85.43	1.11	1.03 a 1.19	
Municipality						
Petrópolis	192	147	76.55	1		
Teresópolis	172	133	77.33	1.01	0.90 a 1.13	0.481
Itaguaí	116	104	89.66	1.17	1.06 a 1.29	0.003
Seropédica	144	123	85.42	1.12	1.01 a 1.24	0.030
Mangaratiba	90	72	80.00	1.04	0.92 a 1.19	0.311
Altitude (m)						
<500	352	300	85.23	1.10	1.01 a 1.20	0.014
500 to <800	163	125	76.69	0.99	0.88 a 1.11	0.487
800 to <1004	199	154	77.39	1		
Farming conditions						
Excellent	127	97	76.38	1		
Good	270	210	77.78	1.02	0.91 a 1.14	0.428
Moderate	134	114	85.07	1.11	0.99 a 1.26	0.052
Poor	183	158	86.34	1.13	1.01 a 1.27	0.018
Area of origin of equids						
Outside the farm	496	390	78.63	1		0.008
Inside the farm	218	189	86.70	1.10	1.03 a 1.18	

N: number of animals examined by indirect fluorescent antibody test (IFAT); n: number of seropositive animals; CI: confidence interval.

and can produce false negative results in chronic infections by *T. equi*. Other studies reported a prevalence of *T. equi* infection similar to that found in the present study (BALDANI et al., 2004; XUAN et al., 2001; HEIM et al., 2007). However, the prevalence of *T. equi* infection reported in southern Brazil is lower than that reported in present study (CUNHA et al., 1996; SOUZA et al., 2000; NIZOLI et al., 2008; GOLYNSKI et al., 2008). This difference may be due to climate influence in the biological cycle of *R. (B.) microplus* in the southern region. Brum et al. (1987) demonstrated three generations of *R. (B.) microplus* per year in the municipality of Pelotas, southern Brazil. However, four generations of this tick have been reported in the southeastern region (MAGALHÃES; LIMA, 1991).

The association of altitude with seropositivity to *T. equi* shows that increasing altitudes can reduce the likelihood of horse infection. This can be explained by climate interference in the cycle of the tick vector at high altitudes. Higher seropositivity rates were seen in equids from inside the farm, reflecting specific characteristics of the farm including inadequate tick control or of other parasites that could reduce the risk of infection. As this is an endemic area for equine theileriosis and of enzootic stability for *T. equi*, horses brought from other regions or even countries may not yet have been infected.

Due to the small number of mules examined compared to horses, the lack of association between *T. equi* infection and animal species should not be generalized until a larger number of mules is investigated in further studies. The lack of association with gender is consistent with other studies (SHKAP et al., 1998; SOUZA et al., 2000; BOTTEON et al., 2002; KOUAM et al., 2010). However, a weak association ($p < 0.047$, OR = 1.498) was reported by Moretti et al. (2010) that found a higher prevalence of anti-*T. equi* antibodies in females compared to males. Similar results were observed in the present study, however, without any significant association.

Foals younger than six months showed the highest frequency of *T. equi* antibodies when compared with older equids (Table 2). This can be explained in part by the presence of maternal antibodies, which can persist for three to five months (DONNELLY et al., 1982; HEUCHERT et al., 1999). Animals aged six to 12 months showed 87.5% (n = 28) of *T. equi* seropositivity. The absence of maternal antibodies in this group suggests that the first *T. equi* infection was between six and 12 months or younger, since there is no passive immunity over six months old. Thereafter, the frequency of antibodies remained stable with no significant increase in animals older than 10 years, 85.42% (n = 170). This may explain why the animals had no clinical signs of equine theileriosis, since once infected with *T. equi*, horses remain carriers throughout their lifetime (FRIEDHOFF, 1988; DE WALL, 1992) and are important reservoirs of this protozoan for tick vectors (UETI et al., 2008). The seroprevalence above 75% seen in animals aged six to 12 months indicate that there exists enzootic stability for *T. equi* in the microregions studied (MAHONEY; ROSS, 1972). The lack of association between age and seropositivity for *T. equi* is consistent with some reports in the literature (SHKAP et al., 1998; SOUZA et al., 2000; BOTTEON et al., 2002; MORETTI et al., 2010). However, other studies found inconsistent results (RIBEIRO et al., 1999; RÜEGG et al., 2007; KOUAM et al., 2010). They could be explained in part by different age categorization. Furthermore, the association of age reported in the literature can be due to small numbers of animals in some categories, which can interfere in the analysis of the data.

Higher frequency of horses seropositive for *T. equi* was seen in those used for exhibition and sports. This can be explained by the stress and animal moving for training and competitions (HAILAT et al., 1997). Among those used for breeding, they may be more exposed to tick infestations since most are raised in extensive or semi-confined systems.

The non-significant increase in seropositivity of the equids reared in semi-confined (75.6%, n = 124) and extensive (13.9%, n = 69) systems can be explained by the fact that these animals have closer contact with cattle (data not shown) and consequently with when compared to those reared in confined systems. *R. (B.) microplus* tick is the only known biological vector that transmit *T. equi* in Brazil (GUIMARÃES et al., 1998) and the U.S. (UETI et al., 2005, 2008). Cattle are the primary host for *R. (B.) microplus*, and infestation of horses with this tick is dependent on the presence of cattle in the same area (LABRUNA et al., 2001). However, the presence of equids with cattle in the same area was not associated with seropositivity for *T. equi*, probably due to the fact that this was an enzootic stability area for this agent. Furthermore, other forms of transmission are possible, such as iatrogenically through infected blood (TENTER; FRIEDHOFF, 1986), transplacentally (ALLSOPP et al., 2007) and congenitally (SANTOS et al., 2008). Bloodsucking flies were also suggested vectors, but without confirmation. Similar findings were reported by Botteon et al. (2002), however, they reported differences ($p < 0.05$) in *T. equi* seropositive horses when reared in confined systems. This difference was not observed in this study, which may be due to the small number of animals evaluated in this group.

The differences in tick parasitism between Itaguaí and Serrana microregions demonstrate their distinct edafoclimatic characteristics, which would influence the epidemiology of equine theileriosis. Evidence of parasitism by three tick species in this study is consistent with the results reported in studies conducted in Brazil (BORGES; LEITE, 1998; HEUCHERT et al., 1999; LABRUNA et al., 2001; DA COSTA PEREIRA et al., 2005; KERBER et al., 2009). Unlike in the present study, an association ($p < 0.001$) between *T. equi* positivity and low infestation by *D. nitens* (mean lower than 10 adult ticks per animal) and high infestation by *A. cajennense* (mean higher than 10 adult ticks per animal) was found (KERBER et al., 2009). This disagreement can be explained by different categorization of degree of infestation. Also, the analysis was grouped by farms rather than by animals as we did in this study. Furthermore, *D. nitens* and *A. cajennense* are not able to transmit *T. equi*, which explains the lack of association with seropositivity of horses seen in present study and reported by Rüegg et al. (2007).

According to Kerber et al. (2009), *A. cajennense* infestation is associated with *T. equi* infection and this tick could be a vector of this agent under natural conditions, though no experimental studies has evidenced that *A. cajennense* is a competent vector. This epidemiological indication is reinforced by the fact that all horses examined were young (6-24 months old), restricted to the farm area and never received blood or plasma transfusions.

In addition, all farms reported the complete absence of any cattle for at least 36 months, which was a period longer than the age of the horses examined. Different from the reported by Kerber et al. (2009), most animals examined in the present study were older than 24 months and many of them have left the farm area for any reason. However, the possibility of *A. cajennense* and even *D. nitens* and *S. calcitrans* act as a vector of *T. equi* cannot be ruled out as it could explain in part the enzootic stability existing in the microregions studied.

Based on the study results, we conclude that *T. equi* infection is more prevalent in the microregion of Itaguaí than Serrana. However, both microregions are endemic areas for equine theileriosis with enzootic stability for *T. equi*. Only factors related to the collection area influenced *T. equi* seropositivity in equids in that region in the state of Rio de Janeiro. These factors should be used for the development of programs for prevention and control of equine theileriosis.

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