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CLINICAL, HEMATOLOGICAL AND RISK FACTORS ASSOCIATED WITH BORRELIOSIS IN HORSES FROM SAO PAULO, BRAZIL

FATORES CLÍNICOS, HEMATOLÓGICOS E DE RISCO ASSOCIADOS À BORRELIOSE EM CAVALOS DE SÃO PAULO, BRASIL

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SUMMARY

Lyme borreliosis is caused by *Borrelia burgdorferi* sensu lato and affects humans and many other mammals, including horses. This disease is poorly studied and reported in horses, and epidemiological surveys are required to provide more precise information about the course of the disease. The aims of the present study were to determine the prevalence of seropositive horses for *Borrelia burgdorferi* sensu lato in São Paulo State, Brazil and to collect data on possible risk factors associated with the disease along with clinical and hematological changes in seropositive horses. There was verified that there was a high correlation between the occurrence of seropositive horses infested with *Amblyomma sculptum* ticks and the presence of capybaras on the property as well as the occurrence of abortion and retained placenta in mares. In terms of hematological alterations, the occurrence of lymphopenia was observed in seropositive animals. Borreliosis in horses from São Paulo, Brazil can be associated with presence of *Amblyomma scupltum* ticks, proximity with capybaras and can be manifested as alterations in reproduction of mares and lymphopenia.

KEY-WORDS: *Borrelia burgdorferi*. *Amblyomma sculptum.* Capybaras. Reproduction. Lymphopenia.

RESUMO

A borreliose de Lyme é causada por *Borrelia burgdorferi* sensu lato e afeta humanos e muitos outros mamíferos, incluindo cavalos. Esta doença é pouco estudada e relatada em equinos, sendo necessários levantamentos epidemiológicos para fornecer informações mais precisas sobre o curso da doença. Os objetivos do presente estudo foram determinar a prevalência de cavalos soropositivos para *Borrelia burgdorferi* sensu lato no Estado de São Paulo, Brasil e coletar dados sobre possíveis fatores de risco associados à doença, juntamente com alterações clínicas e hematológicas em cavalos soropositivos. Verificou-se que houve alta correlação entre a ocorrência de equinos soropositivos infestados por carrapatos *Amblyomma sculptum* e a presença de capivaras nas propriedades, bem como a ocorrência de abortamento e retenção de placenta em éguas. Em termos de alterações hematológicas, observou-se a ocorrência de linfopenia em animais soropositivos. A borreliose em cavalos de São Paulo, Brasil, pode estar associada à presença de carrapatos *Amblyomma scupltum*, proximidade com capivaras e se manifestar clinicamente como alterações reprodutivas em éguas e linfopenia.

PALAVRAS-CHAVE: Borrelia burgdorferi. Amblyomma sculptum. Capivaras. Reprodução. Linfopenia.

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INTRODUCTION

Lyme disease is the most common infectious disease transmitted by ticks to humans and is caused by *Borrelia burgdorferi* spirochetes (Koedel et al., 2015; Udziela et al., 2020). The group of *B. burgdorferi* sensu lato (s.l.) comprises at least 20 genospecies of related bacteria, including *B. burgdorferi* stricto sensu (s.s.), *B. afzelii, B. garinii, B. bavariensis, B. bissetti, B. kurtenbachii, B. lusitaniae, B. chilensis, B. japonica, B. sinica, B. americana* and others (Nava et al., 2014). Most *B. burgdorferi* s.l. species are related to hard ticks of the genus *Ixodes*, especially the *Ixodes ricinus* complex. However, there are reports supporting the occurrence of that *Borrelia spp.* in *Amblyomma americanum* (Clark et al., 2013) and *Dermacentor nitens* (Gonçalves et al., 2013).

Lyme borreliae are carried in the midgut of unfed ticks. When an infected tick acquires a blood meal over at least 18 hours of attachment, the number of spirochetes increases, and the spirochetes express outer surface proteins that support their survival in the vertebrate host. These bacteria migrate from the midgut to salivary glands and are carried into the animal (Stanek et al., 2012).

In humans, acute infection generally causes skin inflammation at the site of a tick bite, referred to as erythema migrans, and may be accompanied by systemic symptoms such as fever, muscle and joint pain, headache, enlargement of the lymph nodes and neurological symptoms (Wasiluk et al., 2011). In horses, a broad spectrum of clinical manifestations has been attributed to *B. burgdorferi* infections including arthritis, lameness, muscle tenderness, anterior uveitis, encephalitis, abortion, foal mortality, low-grade fever and lethargy (Butler et al., 2005; Neely et al., 2020).

The first report of suspected borreliosis in humans from Brazil came from Yoshinari et al. (1989). However, the first case in the country was diagnosed in 1992. An increasing number of identified cases have demonstrated differences between the symptoms described for the Northern Hemisphere disease versus that occurring in Brazil (Yoshinari et al., 1997; Costa et al., 2001). Concerning epidemiology, the occurrence of *Ixodes* ticks is insufficient to allow them to be classified as main vectors in Brazil. Clinically, in addition to the identification of patients with erythema migrans and the usual systemic complications, the Brazilian disease presents many recurrences, autoimmune pathogenesis and challenges in treatment (Mantovani et al., 2007). The spirochete *B. burgdorferi* (s.l.) has been identified in skin biopsies from 22 human patients from Amazonas via immunohistochemistry and focus floating microscopy (Talhari et al., 2010), showing a cystic form without flagelae. Due to those differences, this borreliosis is called Baggio-Yoshinari Syndrome in Brazil (Yoshinari et al., 1997; Silva et al., 2020).

Equine borreliosis is still poorly diagnosed in horses from Brazil, and only three studies have been conducted to describe the disease in native horses. Salles et al. (2002) found that an average of 9.8% of horses from Rio de Janeiro State exhibited anti-*B. burgdorferi* (s.l.) antibodies. Madureira et al. (2007) reported that 28.4% of horses showed seropositivity in Minas Gerais State, and Galo et al. (2009) diagnosed the disease in 26.7% of horses in Pará State. In Campo Grande, Mato Grosso do Sul State, it was verified seropositivity for *B. burgdorferi* in 20.6% in the sampled horses (n=262) (Campos et al., 2021).

The aims of the present study are to present the prevalence of seropositive horses in the cities of São Paulo state with the most registered suspected cases of Lyme disease in humans and to present the main clinical, hematological and risk factors associated with seropositive horses.

METHODS

This study was approved by the University of State of São Paulo (Unesp) Ethics and Welfare Committee (CEUA) in document no. 001968/13.

Epidemiologic assessment

Animal samples

The minimum sample size for the survey of serological prevalence was calculated using the methodology proposed by Thrusfield (2007) to determine the number of horses in groups in two stages (geographic regions and equine properties) with a confidence level of 95%.

The calculation of the minimum number of animals (Ts) followed the model:

$$Ts = \frac{1,96^2.g.P_{esp}(1 - P_{esp})}{g.d^2 - 1,96^2.V_c} \quad (1)$$

Where,

$$V_c = c[\frac{c.V.K_1}{T^2(c-1)} - \frac{K_2.\hat{P}(1-\hat{P})}{T}] \quad (2)$$

$$V = (\hat{P}^2.\text{å}\, n^2) - \{(2.\hat{P}).\text{å}\, nm\} + \text{å}\, m^2 \quad (3)$$

Following,
Ts: minimum animal sample
g: number of groups (cities x properties)
P_{esp}: constant, which is 0.10 for a confidence level of 95%
d: constant error of 0.05
c: number of properties from a previous survey (Salles et al., 2002)
K_1: constant value of 1.0 because the number of sampled groups is far below the number of population groups
K_2: constant value of 1.0 because the number of animals sampled is much lower than the population of the animals
n: number of animals per property (Salles et al., 2002)
m: number of seropositive animals (Salles et al., 2002)
T: sum of n
$\hat{P}$: m/n

The geographic regions to be assessed in the equine survey were determined based on the occurrence of recent (less than 10 years) suspected cases of human Lyme disease diagnosed by the Rheumatology Laboratory of the Medicine Faculty of the University of Sao Paulo. Eleven cities were chosen in Sao Paulo State, and two properties were randomly chosen per city. The number of

horses that was distributed in each city was proportional to the local population of horses and the number of humans suspected of Lyme disease (presence of anti-*B. burgdorferi* immunoglobulins, specific clinical signs and a recent report of a tick bite). Only adult horses were selected for this survey, with their ages varying between 3 and 20 years.

Serology: Indirect ELISA

Immunoglobulins, specifically IgG, against *B. burgdorferi* sensu lato strain G39/40, were detected in accordance with ELISA procedures previously described [20, 21]. Briefly, the preparation of the antigen included sonicating a whole spirochete suspension, which was made from a culture of *B. burgdorferi* organisms grown in a 500 ml bottle containing Kelly's medium at 33ºC at higher-phase growth. The medium was centrifuged at 10,000 g for 20 min at 4ºC, and the pellets were washed 3 times with cold 0.01 M phosphate-buffered saline (PBS) with 5 mM magnesium chloride (pH 7.4). The suspension of spirochetes was sonicated on ice with a cell sonicator, and the supernatant was filtered (45 µm membrane). The protein content was determined by Folin's method, and the antigen preparations were stored in aliquots at -70ºC until further analysis.

Polystyrene plates with 96 holes[3] were coated with antigen at a concentration of 20 mg/mL, incubated in a humidified chamber overnight at 4°C, washed with PBS Tween 20 buffer and then blocked with 1% rabbit serum. The positive control sera were obtained from horses administered 4 serial immunizations over 15 days, and the negative control serum was obtained from 8 healthy horses without history of exposure to ticks. The test and control sera (8 negative and one positive) were diluted 1:800 in PBS Tween 20, incubated and washed. Conjugated rabbit anti-horse IgG linked to alkaline phosphatase[4] was added, incubated and washed. Next, the solution of PNPP[5] diluted in glycine buffer with a pH 10.5 was added, and the samples were read using a spectrophotometer[6] at a wavelength of 405 nm. The cutoff line was established at a confidence level of 99.99%, according to the mean plus three standard deviations of the optical density of the negative controls.

Horse management survey

Concerning the epidemiologic survey, the following questionnaire was presented to the owners:

- Type of stabling: stall (score 0), paddock (score 1) or pasture (score 2),
- Presence of *Amblyomma sculptum* ticks on the horses: present (score 1) or absent (score 0),
- Presence of capybaras (*Hydrochoerus hydrochaeris*) on the property: present (score 1) or absent (score 0).

Medical history assessment

In the same questionnaire, the owners were encouraged to provide information concerning the last 5 years of medical history only for well-known horses. The medical history inquiries included arthritis, myositis, lameness, ataxia, uveitis, abortion, placental retention, back pain and recurrent hemoparasitosis, adopting a score of 1 for presence of the clinical sign and a score of 0 for its absence.

Case-Control clinical trial

Animals

Twenty seropositive horses (Case group) and twenty seronegatives (Control) were chosen for this evaluation. The Case group was composed by adult horses (mares, geldings and stallions aged between 3 and 20 years), in activities of sport or reproduction, from 3 distinct horse farms, that exhibited ELISA titers between 1/400 and 1/3,200 and had been exposed to *Amblyomma sculptum* (Nava et al., 2014) and/or *Dermacentor nitens* ticks. The negative control group was composed by 20 adult horses (mares, geldings and stallions), aged in the same range of Case group, retired or in sport activities, from a single horse farm in which all horses were seronegative due to a severe control of ticks in horses and installations since 2005.

Clinical assessment

All 40 horses were clinically evaluated by a veterinarian. The physical examination included assessment of the color of mucosa, cardiac rhythm, capillary perfusion, respiratory sounds, rectal temperature and dermatological alterations. Qualitative parameters were classified as 0 for normal and 1 for abnormal.

Hematology

Blood samples were collected to evaluate erythrocytes, white blood cells, platelets, bilirubin, creatine phosphokinase, creatinine, gamma glutamyl transferase, total protein, albumin, globulins, aspartate aminotransferase and blood urea nitrogen. The hematological tests were performed with a Poch®-100 IV DIFF automatic analyzer, and biochemical tests were performed with a Roche®COBAS MIRA Plus using Labtest® kits.

Statistical analysis

Nonparametric epidemiological data were verified by Kolmogorov-Smirnov test, analyzed by Chi-Square test at 95% significance and by Cluster Analysis multivariate test using the complete linkage rule and measurement based on Euclidean distances. The case-control parametric data were verified by ANOVA and analyzed with a t-Test with 95% significance and through a multivariate principal component analysis using information on the two major factors. All data were processed using the Statistica® v.12 software (StatSoft Inc., DELL Software).

[3]Bio-Rad Laboratories
[4]Sigma Chemical
[5]Sigma Chemical
[6]BioRad Laboratories, model 550 Microplate Reader

RESULTS

Epidemiologic assessment

The methodology proposed by Thrusfield (2007) resulted in a minimum sample of 651 animals to provide sufficient data to estimate the prevalence of seropositivity among the horses in the 11 cities with registered cases of suspected Lyme disease in humans. In this study, blood samples for serological ELISA tests were collected from 760 horses, distributed among 22 training centers or horse farms, which were randomly chosen. The macro-region (radius of 50 km from the city) formed by the cities of Ribeirão Preto, Jaboticabal and Analândia, located in the center of São Paulo State, presented the highest prevalence rates of seropositivity among horses (Table 1)

Table 1 - Distribution of horses according to their antibody titers against *B. burgdorferi* sensu lato, obtained through ELISA, in the 11 cities of São Paulo state, Brazil, with the most reported cases of suspected Lyme disease in humans.

City	Antibody titer (ELISA)						Horse prevalence	Human cases
	N	0	1/400	1/800	1/1600	1/3200		
São Paulo	10	10	0	0	0	0	0%	5
Sorocaba	30	30	0	0	0	0	0%	9
Bauru	30	28	1	1	0	0	7%	2
Campinas	96	89	4	2	1	0	7%	4
São José do Rio Preto	28	24	3	0	1	0	14%	44
Araraquara	30	25	2	3	0	0	17%	1
São José dos Campos	246	199	36	8	2	1	19%	30
Colina	98	72	18	6	2	0	27%	1
Ribeirão Preto	129	89	27	10	2	1	31%	29
Analândia	17	10	5	2	0	0	41%	1
Jaboticabal	46	24	15	6	1	0	48%	1
Total	760	600	111	38	9	2	21%	127

Considering the total of 760 horses, data about risk factors were obtained regarding the stabling type, presence of ticks in horses and capybaras in the properties, and recent clinical history (past 5 years) of a subgroup of 124 animals, representing approximately 15% of all tested horses. It should be noted that seropositivity for *B. burgdorferi* was directly related to infestation by ticks and the presence of capybaras on the property (Table 2).

Table 2 - Scoring points for the risk and clinical history (5 years) parameters of a subgroup of 124 adult horses (59 seronegatives and 65 seropositives for *B. burgdorferi*), stabled on 22 properties from 11 cities of São Paulo State.

	Seronegatives (n=59)		Seropositives (n=65)		p-value
	Mean	Std. Dev.	Mean	Std. Dev.	
Presence of ticks	1.90	0.80	2.45	0.59	$p < .003$
Presence of capybaras	0.39	0.49	0.83	0.38	$p < .001$
Idiopathic arthritis	0.08	0.28	0.05	0.21	$p > .10$
Recurrent myositis	0.08	0.28	0.00	0.00	$p > .10$
Idiopathic lameness	0.15	0.36	0.03	0.17	$p > .10$
Ataxia	0.08	0.28	0.02	0.12	$p > .10$
Recurrent uveitis	0.03	0.18	0.03	0.17	$p > .10$
Abortion	0.09	0.29	0.15	0.36	$p > .10$
Retained placenta	0.00	0.00	0.05	0.21	$p > .10$
Recurrent hemoparasitosis	0.00	0.00	0.03	0.17	$p > .10$
Back pain	0.03	0.18	0.00	0.00	$p > .10$

Lines in bold show differences between seronegative and seropositive horses according to the Chi-Square test compared to ELISA, with 95% significance.

Multivariate cluster analysis revealed parameters that pointed to a close inter-relationship with the presence of seropositivity, which included the following: ticks, capybaras, abortion and retained placenta, in which constituted Group A. Group B was composed of the type of stabling (stall, paddock or pasture), idiopathic lameness, back pain, arthritis, myositis, ataxia, uveitis and recurrent hemoparasitosis, which showed a weak inter-relationship with seropositive horses (Figure 1)

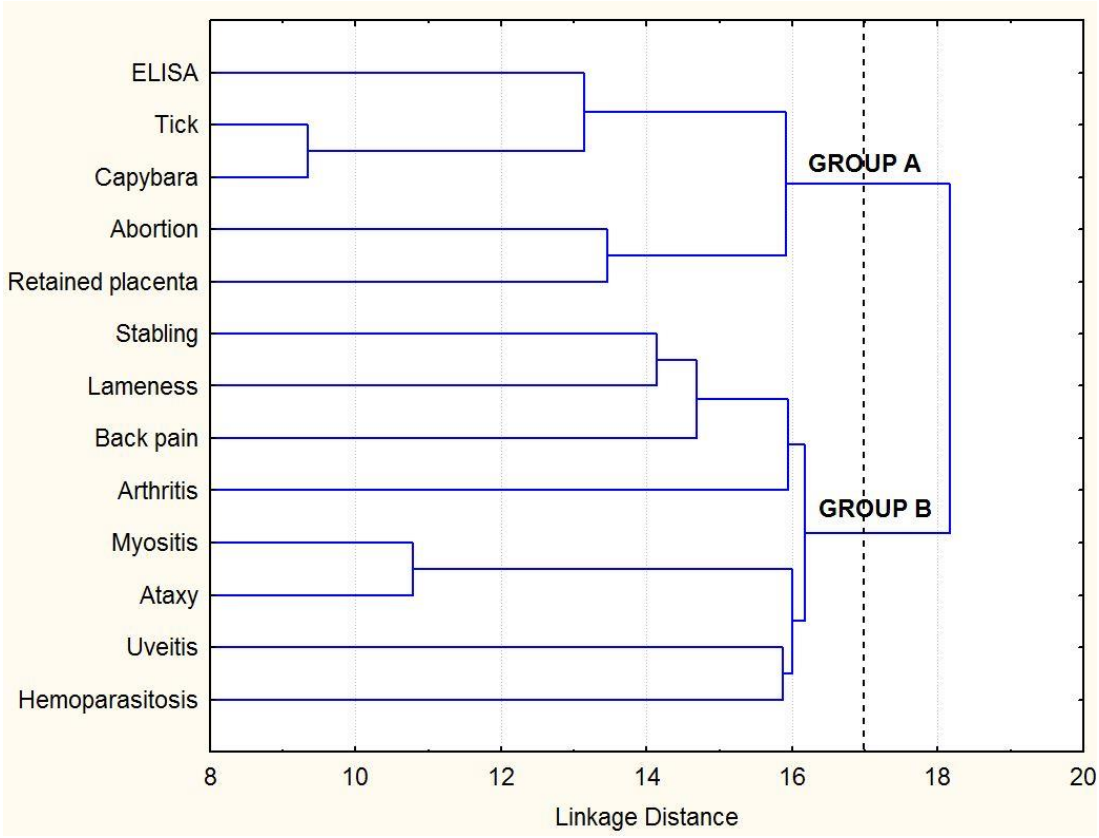

Figure 1 - Cluster analysis of the risk factors and clinical parameters of 124 adult horses (59 seronegatives and 65 seropositives for *B. burgdorferi*) stabled in 22 properties from 11 cities of São Paulo state.

Table 3 presents the difference of occurrence of seropositivity according to the type of stabling of horses. The major cases of seropositive horses were stabled in stalls, when compared with paddocks or pasture. Additionaly, in stalls, it was verified more cases of seropositivity than seronegativity.

Table 3 - Number of cases of seropositivity and seronegativity for *B. burgdorferi* sensu lato in a subgroup of 291 horses from Sao Paulo, Brazil, according to the type of stabling.

ELISA	Stall	Paddock	Pasture	Total
Seronegative	$28^{A,a}$	$59^{B,a}$	$89^{C,a}$	176
Seropositive	$53^{A,b}$	$30^{B,b}$	$32^{B,b}$	115
Total	81	89	121	291

Values in columns followed by capital letters differs each other by Tukey's test with 95% of significance. Values in lines followed by lower case letters differs each other by Tukey's test with 95% of significance.

Case-control clinical trial

No changes in the color of mucous membranes, cardiac rhythm, capillary perfusion, respiratory sounds or rectal temperature or dermatological alterations were recorded in either group of animals. Hematological parameters showed a difference between the groups in terms of the packed cell volume, white blood cells, neutrophils (band and mature), lymphocytes, creatine phosphokinase, creatinine and aspartate aminotransferase, but all of these parameters were within the normal range (Table 4).

Table 4- Hematological parameters of 20 seronegative and 20 seropositive horses in a case-control clinical trial, from São Paulo, Brazil.

Hematological parameters	Seronegatives (n=20)		Seropositives (n=20)		p-value	Reference values (Orsini and Divers, 2013)	
	Mean	Std. Dev.	Mean	Std. Dev.		Min	Max
Red blood cells, $x10^9$/mL	7.5	0.94	7.9	1.24	0.2134	6.8	12.9
Hemoglobin, g/dL	11.3	1.49	12.2	1.58	0.0638	11	19
Packed cell volume, %	34.1	4.39	37.0	4.65	0.0497	32	53
Mean corpuscular volume, fl	45.5	3.02	45.5	7.57	0.9935	37	58.5
Mean corpuscular hemoglobin, pg	15.1	1.06	15.5	1.38	0.2745	12.3	19.9
Mean corpuscular hemoglobin concentration, g/dl	33.1	0.76	33.0	0.44	0.6667	31	38.6
White blood cells, per mL	8730.0	1095.49	9780.0	2029.42	0.0488	5400	14300
Neutrophils (band), per mL	211.7	59.61	396.7	320.85	0.0155	0	100
Neutrophils (mature), per mL	5223.8	859.18	6569.1	1428.74	0.0009	2300	8600
Eosinophils, per µL	210.0	229.58	449.8	496.91	0.0574	0	1000
Lymphocytes, per mL	2880.3	1102.36	2134.5	745.43	0.0166	1500	7700
Monocytes, per mL	204.3	55.55	230.0	76.83	0.2338	0	1000
Basophils, per mL	0.0	0.00	0.0	0.00	1.0000	0	290
Platelets, per mL	168550.0	34841.71	161850.0	32472.30	0.5330	100000	600000
Bilirubin conjugated, mg/dL	0.2	0.05	0.3	0.10	0.0604	0	0.4
Bilirubin unconjugated, mg/dL	0.6	0.22	0.6	0.19	0.9939	0.2	2
Creatine phosphokinase, IU/L	161.2	50.14	259.4	145.31	0.0069	119	287
Creatinine, mg/dL	1.3	0.14	1.1	0.27	0.0168	0.9	1.9
Gamma-glutamyl transferase, IU/L	9.9	2.75	10.1	4.56	0.8676	4	44
Protein (total), g/dL	5.4	0.74	5.7	1.25	0.3730	5.8	8.7
Albumin, g/dL	2.2	0.31	2.1	0.58	0.3461	2.6	3.7
Globulin, g/L	3.2	0.62	3.7	0.82	0.0701	2.6	4
Aspartate aminotransferase, IU/L	290.5	32.01	234.8	64.22	0.0013	226	336
Urea, mg/dL	30.1	6.40	32.8	8.84	0.2757	21	51

Lines in bold show a difference between seronegative and seropositive horses based on a t-Test at 95% significance.

Multivariate analysis of principal components assembled in the same data quadrant, including serology ELISA, creatine phosphokinase, neutrophils, white blood cells, platelets, lymphocytes, monocytes, hematocrit, hemoglobin and erythrocytes (Figure 2), indicating a high degree of inter-relationship between these variables.

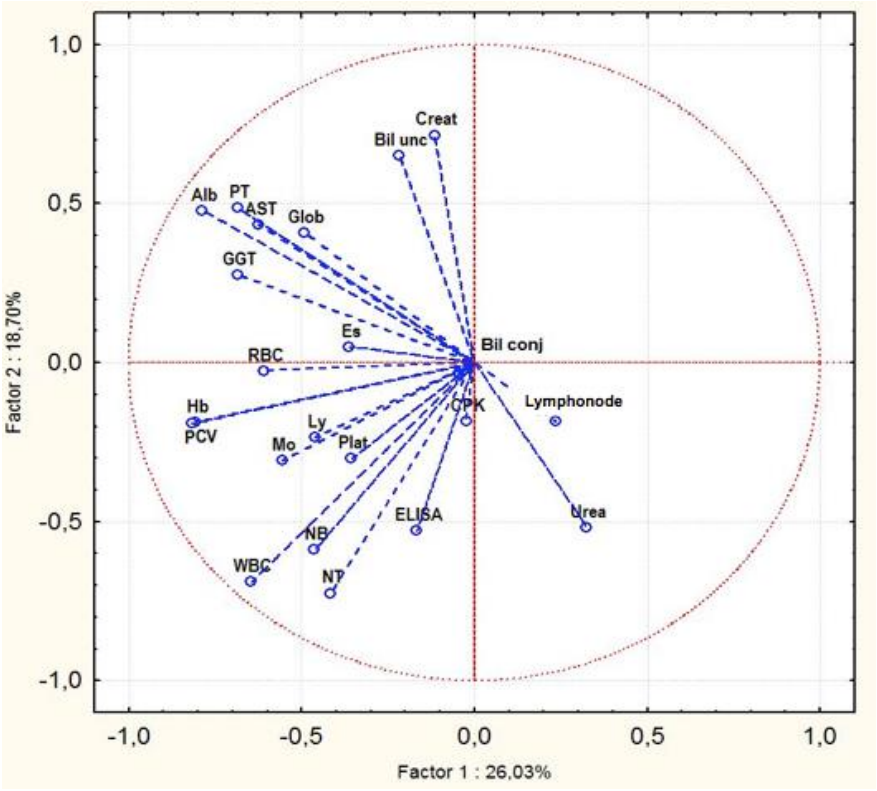

Figure 2 - Principal component analysis of the clinical and hematological parameters of 20 seropositive and 20 seronegative horses (immunoglobulins anti-*Borrelia burgdorferi*). Creat: creatinine, Bil unc: bilirubin unconjugated, Glob: globulins, AST: aspartate aminotransferase, PT: total protein, Alb: albumin, GGT: gamma glutamyl-transferase, Es: eosinophils, RBC: red blood cells, Hb: hemoglobin, PCV: packed cell volume, Mo: monocytes, Ly: lymphocytes, Plat: platelets, WBC: white blood cells, (cont.) (continued) NB: neutrophils (band), NT: neutrophils (mature), ELISA: enzyme-linked immunosorbent assay, CPK: creatine phosphokinase.

DISCUSSION

The findings presented in this study challenge the findings about borreliois in horses published to date. Because of the scarcity of epidemiological studies that have clinically described the disease and its risk factors (Manion et al., 2001; Divers, et al., 2018) and the fact that there are few studies involving experimental infection of horses (Chang et al., 2000; Chang et al, 2005), the clinical signs and risk factors attributed to horses are mostly supported by case reports (Manion et al., 1998; James et al., 2010; Imai et al., 2011; Sears et al., 2011; Priest et al., 2012; Passamonti et al., 2015), resulting in extrapolation of the clinical signs that have been detected in humans (Divers, 2013). These uncertainties may indicate that Lyme borreliosis could be overdiagnosed in horses (Bartol, 2013). In USA, the best-documented naturally occurring syndromes attributed to B. burgdorferi infection in horses include neuroborreliosis, uveitis, and cutaneous pseudolymphoma (Johnstone et al., 2016).

We note an intrinsic relationship between the presence of *Amblyomma sculptum* ticks, proximity to capybaras (*Hydrochoerus hydrochaeris*) and the incidence of seropositivity in the horses on the surveyed properties. Capybaras are the most important group of hosts for all parasitic stages of *Amblyomma sculptum* (Nava et al., 2014; Krawczak et al., 2014) in Brazil, and the southeastern portion of the country comprises an endemic area for the occurrence of both species (Szabó et al., 2013). Although there are no reports of the isolation of *Borrelia burgdorferi* from *Amblyomma sculptum*, a study identified the presence of this pathogen in *Amblyomma americanum* specimens obtained from human patients in the United States (Clark et al., 2013).

In Brazil, DNA fragments of *Borrelia burgdorferi* sensu lato strain B31 have been detected through molecular analysis of *Dermacentor nitens* ticks sampled from equine ears (Gonçalves et al., 2013) in the southern region (Nested Polimerase Chain Reaction – PCR, targeting the 5S (rrf) 23S (rrl) intergenic spacer region, 99,9% of BLAST similarity with *B. burgdorferi* s.s.). Additionally, molecular detection of *Borrelia burgdorferi* sensu lato has been recorded in the blood of human patients from the same area (Nested PCR targeting the 5S (rrf) 23S (rrl) intergenic spacer region, 100% of BLAST

similarity with *B. burgdorferi* s.s.) (Gonçalves et al., 2015).

Multivariate analysis of epidemiological data also showed a relationship between seropositivity and the occurrence of abortion and retained placentas in mares, besides it was not verified the occurrence of another most common diseases like herpes virus (EHV-1) and leptospirosis. This association of *B. burgdorferi* seropositivity and abortion shall be better evaluated in mares. There are reports of abortion associated with borreliosis in cattle (Parker and White, 1992) and humans infected by *Borrelia burgdorferi* sensu lato (Carlomagno et al., 1988; Markowitz et al., 1986; Shapiro, 2014). The exact pathogenesis of the *B. burgdorferi* acquired during pregnancy is undefined. There are doubts about if the bacteria colonize fetuses or placenta causing the negative pregnancy outcomes (Lakos and Solymosi, 2010).

In this study, the major seropositivity was identified in horses stabled in stalls, which contradicts the results reported by Manion et al. (2001), who observed a higher incidence of Borrelia-seropositive horses in pastures. This result is intriguing and suggests the possibility of existence of another vectors in Brazil besides ticks, like arthropods as *Stomoxys calcitrans*, very present in stalls and less frequent in pastures. *Borrelia afzelii* was isolated from *Aedes vexans* (Halouzka et al., 1998) and from *Culex pipiens* (Zakovska et al., 2006) in Czech Republic. The presence of the pathogen in the mosquitoes does not prove their participation in the epidemiological cycle because they are hematophagous, but these results suggest the need of more researches in this field.

Clinical signs that are usually attributed to Lyme disease in horses, such as arthritis, uveitis, ataxia, lameness, myositis and recurrent hemoparasitosis (Divers, 2013) showed no relationship with seropositivity in our survey (Divers et al, 2018).

In the case-control study, despite all of the normal hematological parameters, a difference between leukocyte values was observed, characterized by light neutrophilia and lymphopenia in seropositive horses, in addition to hemoconcentration and a slight increase of creatine phosphokinase. There weres observed signals of leukopenia, monocytopenia and lymphopenia in other recent studies in Brazil (Campos et al., 2021).

The increase in neutrophils can be explained by the innate immune response of the host system in contact with *Borrelia*. Although these bacteria are Gram negative, *B. burgdorferi* do not produce endotoxins (lipopolysaccharides) but express alternative proteins known as outer surface proteins (Osp's). These proteins act as pathogen-associated molecular patterns (PAMPs) that activate the innate immune system via toll-like receptors (TLRs). The recognition mediated by TLRs promotes an immune signaling cascade, involving chemokines, matrix metalloproteinases and a large subset of cytokines, including proinflammatory mediators and neutrophil attractants (Berende et al., 2010; Mason et al., 2014.).

Lymphopenia in *B. burgdorferi* infections is directly associated with the cytopathic mechanisms of these bacteria in T and B-lymphocytes. In an *in vitro* study using *Borrelia burgdorferi* sensu lato and human lymphocytes, it was observed that these spirochetes invade leukocytes approximately 1-2 hours after their first contact. Spirochetes adhere to more than 90% of lymphocytes and form invaginations in their membranes, similar to those formed in the pinocytosis mechanism. Then, the cells are penetrated, and vacuoles were formed. Researchers have observed high mobility of bacteria in these vacuoles, indicating an absence of lysosomal action. Subsequent cell membrane rupture in numerous lymphocytes has been previously reported (Dorward et al., 1997; Campos et al., 2021).

An elevation of creatine phosphokinase has also been reported in humans (Holmgren and Matteson, 2006), and in most cases, only one group of muscles is affected by myositis. This inflammation and sarcomere rupture may be associated with extensive migration of borrelias to the connective tissue, the presence of high plasmocellular infiltration and degeneration of fibers (Reimers et al., 1993).

CONCLUSION

Epidemiological surveys can contribute to existing knowledge of the risk factors, clinical signs and pathogenesis of diseases. For equine Lyme borreliosis in particular, many clinical signs that have been associated with horses due to their occurrence in humans can be misinterpreted. Brazilian borreliosis still presents an obscure pathogenesis in horses, but this study shows that *Borrelia* should have an impact on the reproductive tract of mares. Capybaras and *Amblyomma sculptum* can play an important role in the biological cycle of *Borrelia burgdorferi* in São Paulo State, Brazil and their transmission to horses can be associated to another vectors besides ticks.

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