



# Tick-borne zoonotic agents infecting horses from an urban area in Midwestern Brazil: epidemiological and hematological features

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## Abstract

The emergence of tick-borne diseases has been reported as a serious problem in public health worldwide and many aspects of its epidemiology and effects on the health of its hosts are unclear. We aimed to perform an epidemiological study of tick-borne zoonotic *Rickettsia*, *Borrelia*, and Anaplasmataceae in horses from Midwestern Brazil. We also evaluated whether *Borrelia* spp. and Anaplasmataceae may be associated with hematological disorders in the sampled animals. Blood and serum samples as well as ticks were collected from 262 horses. Serum samples were used to perform serological tests, and hematological analyses were made using whole blood. Furthermore, DNA extracted from whole blood and ticks was used for molecular tests. Campo Grande is enzootic for tick-borne studied bacteria, since we found an overall exposure of 59.9% of the sampled horses, 28.7% of them presented co-exposure. Seropositivity rates of 20.6% for *Borrelia* spp., 25.6% for *Rickettsia* spp., and 31.6% for Anaplasmataceae were found in the sampled horses. Considering both molecular and serological tests for *Borrelia* spp., the infection rate was 48.0% (126/262). None of the tested horses showed molecular positivity for *Anaplasma phagocytophilum*. The horses sampled displayed 7.2% of parasitism by ixodid ticks in single and coinfections. We did not find DNA of any studied bacteria in the sampled ticks. Positive horses for *Borrelia* spp. and Anaplasmataceae agents displayed leukopenia, monocytopenia, and lymphopenia. Together, our results suggest that horses may play a role as sentinel host for zoonotic bacteria and *Borrelia* spp. and Anaplasmataceae agents can impair the health of horses.

**Keywords** Anaplasmataceae · *Borrelia* spp. · *Rickettsia* spp. · Sentinel hosts · Ticks

## Introduction

Horses have been reported infected with *Anaplasma phagocytophilum* and exposed to *Borrelia burgdorferi* and *Rickettsia rickettsii*, etiological agents of human granulocytic anaplasmosis (HGA), Lyme borreliosis (LB), and Brazilian spotted fever (BSF) in Brazil, respectively (Souza et al., 2016; Socoloski et al., 2018; Dos Santos et al., 2019). Ixodid tick vectors play an important role in the maintenance of these bacteria in endemic areas through efficient transmission mechanisms, such as transovarian transmission in the case of *Rickettsia* spp. (Piranda et al., 2011) and transstadial perpetuation in the cases of *A. phagocytophilum* and *Borrelia* spp. (Pusterla et al., 2002; Cordeiro et al., 2018). The role of ticks in the transmission of *B. burgdorferi* sensu lato (s.l.) or *A. phagocytophilum* (previously known as *Ehrlichia equi*) in Brazil remains unproven (Abel et al., 2000; Salvagni

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et al., 2010), in contrast to *Ixodes* sp. ticks in the northern hemisphere (Franke et al., 2013; Dugat et al., 2015). The species *Rhipicephalus (Boophilus) microplus* has been registered as a vector of *Borrelia theileri* to cattle and horses in the Brazilian territory, maintaining this bacterium by transovarian transmission (Yparraguirre et al., 2007; Cordeiro et al., 2018). *Rickettsia rickettsii*, the agent of BSF, is transmitted by *Amblyomma sculptum*, *Amblyomma aureolatum*, and *Rhipicephalus sanguineus* (s.l.) (De Oliveira et al., 2019).

In the state of Mato Grosso do Sul (MS), Midwestern Brazil, serological and molecular evidences of *B. burgdorferi* (s.l.) have been reported in humans (Resende et al., 2016; Lopes et al., 2017). Furthermore, the occurrence of *Rickettsia* species in the state of MS has been recorded in small wild mammals and in large wild rodent capybara (*Hydrochoerus hydrochaeris*) that have been reported as amplifying hosts for *R. rickettsii* (Souza et al., 2009), *Amblyomma* spp., *R. sanguineus*, and humans (De Almeida et al., 2013; Matias et al., 2015; Binder et al., 2017; De Sousa et al., 2018). Serological evidence of exposure to *A. phagocytophilum* was reported in Brazilian marsh deer (*Blastocercus dichotomus*) in the state of MS. Additionally, 16S rRNA Anaplasmataceae genotypes have been detected in dogs, coatis (*Nasua nasua*), and *A. sculptum* ticks in the Pantanal region, MS (Sacchi et al., 2012; André, 2018).

As horses do not present persistent bacteremia of *A. phagocytophilum* and *B. burgdorferi* (s.l.) (Burbelo et al., 2011; Socoloski et al., 2018; Dos Santos et al., 2019) and in the case of *R. rickettsii*, these animals are considered refractory to infection (Ueno et al., 2016), infected or exposed horses by these bacteria have been designated as sentinel hosts in endemic areas (Souza et al., 2016; Socoloski et al., 2018; Dos Santos et al., 2019). The aim of this study was to investigate the role of horses and their related ticks in the epidemiology of these zoonotic tick-borne bacteria in Campo Grande, MS, Midwestern Brazil. The association between serological exposure and/or molecular positivity for the selected agents with hematological parameters in infected horses was also assessed.

## Methods

### Site collection and field procedures

This study was performed in the municipality of Campo Grande, MS, Midwestern Brazil. Between November 2017 and April 2018, whole blood, serum samples, and ticks were collected from 262 horses that had been raised in that region. The animals were maintained in the facilities of the Jockey Club, equestrian riding school, and military police.

Sampling was carried out by the non-probabilistic method (Stevenson, 2005).

### Identification of collected ticks

Ticks parasitizing horses were collected after a visual inspection of 60 s and identified using previously published dichotomous keys (Barros-Battessti et al., 2006; Nava et al., 2014).

### Hematological analysis

Horse blood samples were collected in tubes with and without ethylenediamine tetra-acetic acid (EDTA). In the first 6 h after blood collection, the packed cell volume (PCV) was quantified using microhematocrit technique; the red blood cells (RBC) and white blood cells (WBC) were counted using an automated hematology analyzer (pocH-100iV Diff Veterinary Hematology Analyzer, Sysmex®). The determination of total plasma proteins (TPP) was performed by manual refractometry, and fibrinogen (FIBRI) was assessed by heat precipitation technique in a water bath at 57 °C for 3 min (Kaneko et al., 1997). The monocytes, lymphocytes, neutrophils, eosinophils, and basophilic counts were performed by counting 100 leukocytes in a blood smear stained by the rapid method (Fast Panotic LB®), under light microscopy (1000×).

We used the values of RBC, PCV, hemoglobin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) as indicators for anemia rates; monocyte and neutrophil counts as indicators for infection responses; lymphocyte count as indicators of immune investment; and fibrinogen and platelet count as indicators of coagulation (Kaneko et al., 1997; Parreira et al., 2016).

### Indirect enzyme-linked immunosorbent assay (ELISA) for detection of anti-*B. burgdorferi* antibodies

Serum samples collected from horses were subjected to an ELISA assay using *B. burgdorferi* sensu stricto (s.s.) antigen G39/40 strain diluted to 20 mg/mL according to Socoloski et al. (2018). The sensitivity of the assay ranged from 82.6 to 100% in the analyses for IgG antibodies, according to Magnarelli et al. (1994).

The horse sera used as positive and negative controls (Socoloski et al., 2018) belonged to the serum bank of the Laboratory of Parasitic Diseases at the Federal Rural University of Rio de Janeiro (LDP-UFRRJ). Twelve negative controls and two positive control sera were used per plate. The cutoff point value was calculated based on the *t*-Student distribution with a 99.99% confidence interval, determined with the mean of the negative controls plus three times the

standard deviation of the optical density (OD) values of the negative controls of each reaction. Still, to correct the OD of each plate, the cutoff value was equal to 100, following the formula: (test serum OD  $\times$  100 / cutoff). The results of OD of the sera of the animals tested in the present study were expressed following the formula proposed by Frey et al. (1998) as an optical density index (ODI).

### Indirect immunofluorescence antibody test (IFAT) for detection of IgG antibodies against *Rickettsia* spp. and *A. phagocytophilum*

In order to detect IgG antibodies to *Rickettsia* spp. of the spotted fever group (SFG), slides containing crude antigens derived from three *Rickettsia* isolates from Brazil—*R. rickettsii* strain Taiacu, *R. parkeri* strain At24, and *R. amblyomantis* strain Ac37—were used (Labruna et al., 2004; Pinter and Labruna, 2006; Silveira et al., 2007). On each slide, previously determined non-reactive and reactive serum samples to SFG rickettsiae functioned as negative and positive controls, respectively. The slides were incubated with fluorescein isothiocyanate labeled anti-horse IgG (Sigma®-Aldrich, St. Louis, USA) and examined under a fluorescence microscope (Olympus®, Tokyo, Japan). The *Rickettsia* species identification and cutoff (1:64) were determined according to Horta et al. (2007).

Aiming to detect anti-*A. phagocytophilum* IgG antibodies, we used antigen (Webster strain, kindly supplied by Dr. John Stephen Dumler, from Uniformed Services University of the Health Sciences) obtained from HL-60 cellular culture in the Immunoparasitology Laboratory of University Estadual Paulista, UNESP Jaboticabal, São Paulo, Brazil. Positive and negative Chilean equine serum samples for *A. phagocytophilum* were used as controls (Hurtado et al., 2020). The cutoff of the test was 1:64.

### DNA extraction

DNA extraction from the EDTA whole blood of horses was performed using the phenol chloroform protocol (Sambrook et al., 1989). For tick DNA extraction, the GT (Guanidine) protocol, as adapted by Sangioni et al. (2005), was performed. DNA concentration and absorbance ratio (260/280 nm) were measured using a spectrophotometer (Nanodrop, Thermo Fisher Scientific, USA).

### Conventional PCR assays

To detect *Borrelia* sp. DNA, a conventional polymerase chain reaction (cPCR) was performed using primers that amplify a 500-bp fragment of the coding region of the conserved 23S rRNA gene: Fwd (5'-TAC CCA GCA CTT ACC CTT GG-3') and Rev (5'-AGT GCC AGG TGG GTA GTT

TG-3') (National Institute of Industrial Property—INPI—Case BR 10 2016 021,522 6). The DNA samples used as positive controls were isolated from *B. burgdorferi* (s.s.) strain G39/40 provided by Dr. Matheus Dias Cordeiro from LDP-UFRRJ. The PCR reaction was conducted according to Sal et al. (2008). For the detection of *Anaplasma* spp. in horses, a nested PCR assay (nPCR) was performed using primers directed to the 16S rRNA gene to amplify 932 bp and 546 bp, according to Massung et al. (1998). *A. phagocytophilum* DNA used as positive control was kindly provided by Prof. Dr. John Stephen Dumler (Uniformed Services University of the Health Sciences, Bethesda, MD, USA).

The molecular testing of horses' ticks was performed using the rickettsial *ompA* gene (Regnery et al., 1991; Roux et al., 1996), and DNA of the *R. parkeri* strain NOD was used as positive control (Labruna et al., 2007) (for primer list, see Supplementary File 1: Table S1). Sterile ultrapure water was used as a negative control. We did not perform molecular tests for *Rickettsia* spp. from horses' blood samples due to the absence of rickettsemia in these animals, when experimentally infected with *R. rickettsii* (Ueno et al., 2016).

### Real-time PCR assay for *A. phagocytophilum*

To detect *A. phagocytophilum*, a quantitative polymerase chain reaction (qPCR) assay was performed, using primers: Fwd (5'-AGT TTG ACT GGA ACA CAC CTG ATC-3') and Rev (5'-CTC GTA ACC AAT CTC AAG CTC AAC-3') and probe [FAM] TTA AGG ACA ACA TGC TTG TAG CTA TGG AAG GCA [TAMRA] directed to the coding region of the *msp-2* gene, according to Drazenovich et al. (2006). Plasmids (IDT psmart, Integrated DNA Technologies®) containing the target sequences and diluted from 10<sup>7</sup> to 10<sup>0</sup> were used as positive controls. The negative control used in the reaction was sterile ultrapure water.

### Purification, sequencing, and BLAST analysis of the obtained amplicons

The products of *Borrelia* sp. 23S rRNA amplicons were purified by enzymatic treatment with ExoProStar™ (GE Healthcare), in accordance with the manufacturer's recommendations. The amplicons were sequenced in both directions using an automated system based on the dideoxynucleotide chain-termination method (Sanger et al. 1977).

The quality of the electropherograms obtained through the sequencing of the amplified products was visually analyzed using the program FinchTV 1.4.0. In addition, consensus sequences were obtained by analysis of the forward sequences using the CAP3 program (<http://mobile.pasteur.fr/cgi-bin/MobylePortal/portal.py>). In order to perform sequence identity analysis, the amplified gene

fragments were compared with those previously deposited in GenBank using the Basic Local Alignment Search Tool (BLASTn) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Finally, the obtained sequences were individually aligned with other sequences available from GenBank using ClustalW (Thompson et al., 1994) and adjusted with BioEdit 7.0.5.3 (Hall, 1999).

### Statistical analysis

Descriptive statistic (mean and confidence interval) was applied to obtain the mean of hematological variables. The Shapiro–Wilk test served to establish whether the distribution was normal. Since horses are refractory to *Rickettsia* spp., seropositive animals were not included in hematological analysis. Therefore, a Kruskal–Wallis test was applied to determine the differences among negative animals, and *Borrelia* sp.– and *Anaplasma* sp.–positive animals. Post hoc Mann–Whitney tests were used to assess pair-wise results of the Kruskal–Wallis test. Finally, the Spearman correlation test was performed to assess whether there was a significant association between the seropositivity of the three bacteria studied, as well as among the seropositivity for *Rickettsia* spp. and *A. phagocytophilum* and molecular positivity for *Borrelia* spp. in the sampled horses.

The determination of the influences of exposure to *A. phagocytophilum* and positivity for *Borrelia* spp. in relation to anemia rates, infection responses, immune investment, coagulation, and TPP was carried out by path analysis. To reduce dimensions of anemia rates, infection responses, immune investment, and coagulation values, we used the principal coordinate analysis (PCA), a geometric technique that converts a matrix of distances between points in multivariate space into a projection that maximizes the amount of variation along a series of orthogonal axes. We used an  $r$  value  $\geq 0.60$  to interpret the results (positive or negative effect) of the path analysis. The variables were statistically significant for its  $p$  values  $\leq 0.05$ . All data were analyzed using R 3.6.1 (R Development Core Team, 2015).

### Results

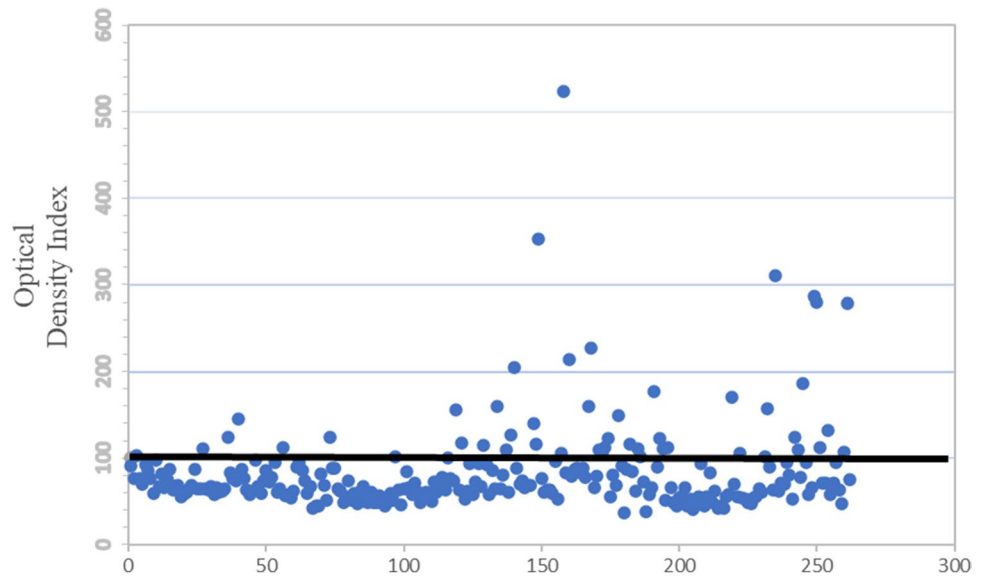
We found that 25.6% (67/262) of the sampled horses were seropositive for *Rickettsia* spp. Among them, 19.8% (52/262) showed seropositivity for *R. rickettsii*, 16.7% (44/262) for *R. parkeri*, and 17.5% (46/262) for *R. amblyommatis* (Table 1). Moreover, 75.0% of seropositive horses for *R. rickettsii* and *R. parkeri* showed serological titers lower than 1:256 (Table 1). A seroprevalence of 31.6% (83/262) was recorded for *A. phagocytophilum*, high number of the seropositive animals (85.4%) with low serological titers (1:64 and 1:128) (Table 1). The search for anti-*B. burgdorferi* (s.l.) antibodies

**Table 1** Seropositivity of horses ( $n = 262$ ) sampled in Campo Grande, Midwestern Brazil

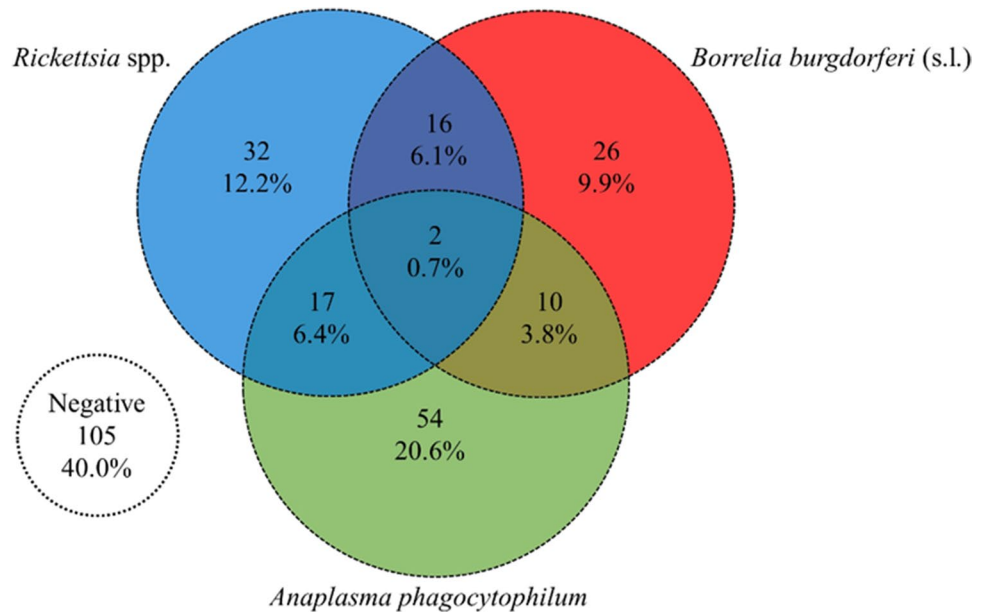
Bacteria	Serological titers						Total
	1:64	1:128	1:256	1:512	1:1024	1:2048	
<i>Rickettsia rickettsii</i>	15 (28.8)	24 (46.1)	10 (19.2)	2 (3.8)	1 (1.9)		52 (19.8)
<i>Rickettsia parkeri</i>	16 (36.3)	17 (38.6)	7 (15.9)	4 (9.0)			44 (16.7)
<i>Rickettsia amblyommatis</i>	11 (23.9)	20 (43.4)	8 (17.3)	6 (13.0)	1 (2.1)		46 (17.5)
<i>Anaplasma phagocytophilum</i>	55 (66.2)	16 (19.2)	3 (3.6)	4 (4.8)	2 (2.4)	1 (1.2)	83 (31.6)

Number of seropositive horses followed by percentage of occurrence of *Rickettsia rickettsii*, *R. parkeri*, *R. amblyommatis*, and *Anaplasma phagocytophilum*. Samples were collected between November 2017 and April 2018

**Fig. 1** Distribution of the optical density indexes of the test sera in relation to the cutoff point (DO×100 / cutoff point [black line]) obtained from the indirect ELISA assay for anti-*B. burgdorferi* antibodies in horses sampled between November 2017 and April 2018, in Campo Grande, Midwest Brazil



**Fig. 2** Three-way Venn diagram illustrating single and co-reactivity for *Anaplasma phagocytophilum*, *Borrelia burgdorferi* (s.l.) and *Rickettsia* spp. in 262 horses sampled in Campo Grande, Midwest Brazil, between November 2017 and April 2018. Data were expressed by the total numbers followed by the percentage of occurrence



revealed that 20.6% (54/262) of the sampled horses were reactive. Although the optical density index ranged from 36.30 to 522.91, they were low for most seropositive horses (Fig. 1). Overall, 59.9% (157/262) of the sampled horses were seropositive for vector-borne agents, in single ( $n=112$ ) and co-exposure ( $n=45$ ) (Fig. 2).

The molecular test showed positivity of 32.4% (85/262) for *Borrelia* spp. Some amplified samples were sequenced and deposited in the GenBank database with access numbers MN539985, MN539986, and MN539988. Sequence analyses revealed 100% sequence identity with *Borrelia* spp. The sequences detected in the present study showed 100% query coverage. Considering the positivity in the molecular and serological tests for *Borrelia* spp., we found the occurrence

**Table 2** Patterns of *Borrelia* sp. infections in horses ( $n=262$ ) from Campo Grande, Midwestern Brazil

Patterns of <i>Borrelia</i> spp. infection	Number of horses
PCR + ELISA + (patent infection)	13/262 (4.9%)
PCR + ELISA – (early infection)	72/262 (27.4%)
PCR– ELISA + (sub-patent infection)	41/262 (15.6%)
PCR– ELISA – (negative)	136/262 (51.9%)

Samples were collected between November 2017 and April 2018. Values are expressed by number of animals, followed by percentages



rate of 48.0% (126/262). Table 2 shows the patterns of *Borrelia* sp. infection in horses in Campo Grande, MS. None of the horses sampled showed molecular positivity for *Anaplasma* spp. and *A. phagocytophilum* test.

Tick infestations were observed in 7.2% (19/262) of the sampled horses, by *Amblyomma sculptum* (5 males and 3 females), *Dermacentor nitens* (16 males and 16 females), and *Rhipicephalus (Boophilus) microplus* (5 males and 32 females) and immature forms of *Amblyomma* spp. (1 nymph), *D. nitens* (36 nymphs), and *R. (B.) microplus* (19 nymphs). Among horses that presented positivity for *Borrelia* spp. ( $n = 126$ ), we observed that six (7.6%) were infested by adults of *A. sculptum* and *R. (B.) microplus*, and *Amblyomma* sp. and *R. (B.) microplus* nymphs. Furthermore, in horses seropositive for *Rickettsia* spp. and *A. phagocytophilum*, we found, respectively, ten (14.9%) and six (7.2%) infested by adults and immature forms for *A. sculptum*, *D. nitens*, and *R. (B.) microplus*. The molecular tests performed in the sampled ticks did not show the presence of the DNA of any vector-borne bacteria addressed in the present study.

The hematological mean values of horses infected and coinfecting with *A. phagocytophilum* and *Borrelia* spp., as well as negative animals, are shown in Table 3. The hematological indexes of sampled horses showed that *A. phagocytophilum*-seropositive horses displayed significant decrease of WBC. Moreover, animals that displayed positivity in the molecular and serological tests for *Borrelia* spp. and co-exposed to *A. phagocytophilum* displayed a significant decrease of monocyte count. In addition, positive horses by *Borrelia* spp. and exposed and co-exposed with *A. phagocytophilum* had a significant increase of MCV values.

Path analysis showed a positive influence of (i) *A. phagocytophilum* seropositivity (path coefficient = 0.18,  $p < 0.05$ ); (ii) horses positive by *Borrelia* spp. (path coefficient = 0.13,  $p < 0.05$ ); and (iii) serological exposure to *A. phagocytophilum* and positivity to *Borrelia* spp. (path coefficient = 0.16,  $p < 0.05$ ) on anemia indicator, expressed by direct increase of MCV ( $r = 0.90$ ) (Fig. 3). Additionally, our results showed that horses seropositive for *A. phagocytophilum* and positive to *Borrelia* spp. (path coefficient = 0.15,  $p < 0.05$ ) were negatively influenced on immune investment expressed by decrease of lymphocyte count ( $r = -0.94$ ) (Fig. 3).

Furthermore, *Borrelia* sp. positivity (path coefficient = 0.16,  $p < 0.05$ ) and seropositivity for *A. phagocytophilum* and *Borrelia* spp. (path coefficient = 0.17,  $p < 0.05$ ) resulted in the negative influence on the infection response, expressed by decreased monocyte count ( $r = -0.79$ ). Also, *A. phagocytophilum* with *Borrelia* spp. (path coefficient =  $-0.14$ ,  $p < 0.05$ ) had direct positive influence on TPP ( $r = -0.98$ ) (Fig. 3).

## Discussion

Our results indicate that horses are constantly exposed to tick-borne bacteria in Campo Grande, Midwestern Brazil, due to seropositivity of 59.9%, in single and multiple exposures. In this sense, horses can be considered sentinel hosts for the risk of infection by tick-borne bacteria to humans.

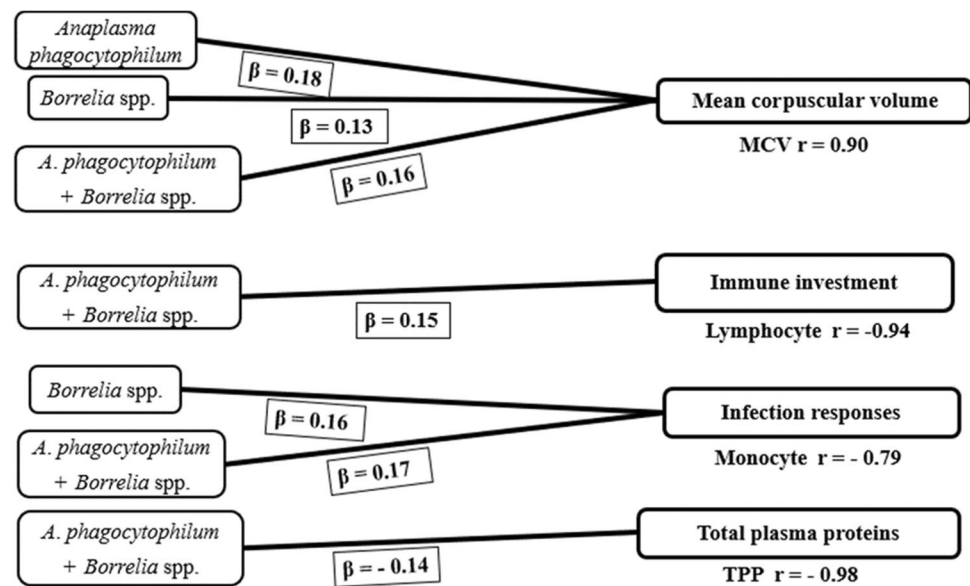
A general seroprevalence for *Rickettsia* spp. of 25.6% (67/262) in sampled horses, even in low serological titers, and of 88.2% (15/17) for tested capybaras (data not shown),

**Table 3** Hematological mean values of horses infected and coinfecting with *Anaplasma phagocytophilum* and *Borrelia* spp. in the Campo Grande – Mato Grosso do Sul, between November 2017 and April 2018

Hematological values	Negatives ( $n = 88$ )	<i>Anaplasma phagocytophilum</i> ( $n = 43$ )	<i>Borrelia</i> spp. ( $n = 84$ )	Coinfection ( $n = 35$ )
RBC $10^6/\mu\text{L}$	8.0 (5.9–10.6)	7.8 (6.0–10.1)	7.8 (4.8–10.3)	7.9 (6.7–10.0)
Hemoglobin g/dL	12 (9–15)	12 (10–15)	12 (8–14)	12 (10–14)
PCV %	36 (27–47)	37 (27–45)	36 (25–45)	37 (31–43)
MCV fL	45 (39–52) <sup>a</sup>	47 (42–50) <sup>b</sup>	46 (39–52) <sup>b</sup>	47 (40–52) <sup>b</sup>
MCHC g/dL	33 (31–34)	33 (32–35)	33 (31–35)	33 (31–34)
TPP g/dL	7 (6–8)	8 (6–12)	7 (6–11)	8 (6–13)
FIBRI mg/dL	3 (0–1)	24 (0–200)	18 (0–293)	69 (0–800)
Platelets $10^3/\mu\text{L}$	139,875 (58,175–225,650)	136,163 (46,300–230,000)	133,238 (27,150–225,950)	130,343 (18,200–225,900)
WBC $10^3/\mu\text{L}$	9730 (5653–14,578) <sup>a</sup>	8398 (5425–11,475) <sup>b</sup>	8935 (5553–12,193)	8754 (5655–14,200)
Neutrophils $10^3/\mu\text{L}$	5256 (2962–9867)	4659 (2980–6783)	5063 (3024–7917)	5054 (2753–9308)
Lymphocytes $10^3/\mu\text{L}$	3802 (1318–8123)	3157 (1077–4927)	3319 (1454–6124)	3117 (1282–5741)
Monocytes $10^3/\mu\text{L}$	289 (0–1133) <sup>a</sup>	228 (0–766) <sup>a</sup>	177 (0–1011) <sup>b</sup>	133 (0–567) <sup>b</sup>
Eosinophils $10^3/\mu\text{L}$	342 (0–1567)	266 (0–979)	334 (0–1731)	328 (0–1004)
Basophils $10^3/\mu\text{L}$	38 (0–308)	38 (0–180)	40 (0–235)	41 (0–206)

RBC, red blood cell counts ( $\times 10^6$ ); PCV, packed cell volume; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; TPP, total plasma protein; WBC, white blood cell counts. Different letters denote statistical significance ( $p < 0.05$ )

**Fig. 3** Schematic representation of some hematological parameters with single and multiple infection patterns among tick-borne agents in horses sampled in Campo Grande, Midwest Brazil, between November 2017 and April 2018



suggests the circulation of rickettsial agents in the studied area. Moreover, our results showed that horses were exposed to different SFG rickettsiae, because of their serological reactivity to three species: *R. rickettsii* (19.8%), *R. parkeri* (16.7%), and *R. amblyommatis* (17.5%). These data, together with Alves et al. (2014), strengthen the occurrence of these rickettsial agents in the central west region of Brazil. Although we observed low infestations by *A. sculptum* in horses, probably due to good sanitary management to which the animals were submitted, we highlight the presence of *A. sculptum* because it is considered the main vector for *R. rickettsia*. Indeed, this tick species is found parasitizing capybaras (Labruna et al., 2011; Szabó et al., 2013), the largest rodent extremely common in forest fragments in the CG that develop high rickettsia, and has anthropophilic habits (Souza et al., 2009; Polo et al., 2017; Ramírez-Hernández et al., 2020).

In the present study, we observed exposure to *A. phagocytophilum* in 31.6% (83/262) of the collected horses, and nine seropositive animals showed high serological titers (1:512 to 1:8192). However, serological tests among Anaplasmataceae agents should be analyzed with caution, as the occurrence of cross-reactivity between species of *Anaplasma* and *Ehrlichia* has been reported in horses (Rolim et al., 2015; Vieira et al., 2016) and jaguars (Widmer et al., 2011). Additionally, we did not detect DNA of the *A. phagocytophilum* in the seropositive animals. In fact, the appropriate molecular target for *A. phagocytophilum* still represents a challenge for the diagnosis because genetic diversity according to their geographical distribution has been reported (De La Fuente et al., 2005; Silaghi et al., 2011). Herein, we used both 16S rRNA nested PCR and *msp-2* qPCR assays to screen the sampled horses for *Anaplasma* spp. Furthermore, experimental infection by *A. phagocytophilum* in horses showed that antibodies

may persist for up to 300 days after inoculation, indicating that horses play a potential role as a sentinel of infections to human (Franzén et al., 2005, 2009). The presence of antibodies to *A. phagocytophilum* might represent a serological cross-reaction to *Ehrlichia* spp. previously detected in horses from Brazil (Vieira et al., 2016) or to a new Anaplasmataceae agent yet to be described.

The occurrence of *B. burgdorferi* in Brazilian territory still remains unclear due to the lack of isolation. Only molecular evidence in humans and a serological study in horses were reported (Resende et al., 2016; Lopes et al., 2017; Socoloski et al., 2018). Since *B. theileri* and *R. (B.) microplus* have been reported parasitizing bovines, ovine, and horses, in Brazil (Soares et al., 2000; Yparraguirre et al., 2007; Cordeiro et al., 2018), the seroprevalence of 20.6% found here may be due to cross-reactions with different species related to recurrent fever group (RFG) and cannot be ruled out (Magnarelli et al., 1994; Rogers et al., 1999). In fact, we found low rates of optical density for most seropositive horses. Moreover, the DNA detected in sampled horses may be related to infection by *B. theileri* since (i) bacteremia during *B. burgdorferi* infection is related as absent or brief (Chang et al., 2000; Maraspin et al., 2011; Talagrand-Reboul et al., 2020), (ii) the primers used here do not distinguish *Borrelia* species, and (iii) we found *R. (B.) microplus* infesting horses. Lack of molecular positivity for *Borrelia* spp. in the sampled ticks is expected because its detection has been recorded as negative (Montandon et al., 2014). Besides, the presence of DNA of *Borrelia* spp. in 32.3% of sampled horses suggests that some reservoir mammal species must be participating as amplifying hosts for *Borrelia* spp. (Radolf et al., 2012) in the studied area. The combination of both molecular and serological tests may help to understand the different epidemiological patterns in a given host population.

For example, the occurrence of 57.1% (72/126) of positive horses with early infection reinforces the enzootic character of the studied area. Additionally, the presence of 32.5% of positive horses (41/126) with a sub-patent infection strongly suggests that seropositive horses that displayed negativity in the molecular test fail in the transmission of tick-borne agents.

The leukopenia recorded in the present study could probably be associated to lysis of infected cells. Furthermore, our analysis showed that positivity of horses by both *Borrelia* spp. and Anaplasmatocaeae resulted in negative influences on the response to infection as well as immune investment, expressed by monocytopenia and lymphopenia, respectively. The decrease of leucocytes was also observed in naturally equine granulocytic anaplasmosis by Gribble (1969) and McQuiston et al. (2003), and lymphopenia was also reported in equines experimentally infected with *A. phagocytophilum* by Franzén et al. (2005). On the other hand, the resultant of infections may present differently as shown by Saleem et al. (2018) that recorded leukocytosis due to lymphocytosis and monocytosis in horses naturally infected by *A. phagocytophilum*. The hyperproteinemia found in horses that had contact to both *Borrelia* spp. and Anaplasmatocaeae in the present study suggests an increase in the production of immunoglobulins. When considered separately, *A. phagocytophilum* naturally infected horses did not display hyperproteinemia (Siska et al., 2013).

The interpretation of hematological results under natural infections should be done with caution whenever the kinetics of infection and the presence of other unrelated agents are unknown. However, comparing negative with positive sampled horses, we found an increase of MCV, indicating macrocytic anemia, as a result of vitamin B<sub>12</sub> deficiency (Satué et al., 2012). As the sampled animals had low fiber diet, and vitamin B<sub>12</sub> is naturally produced in the large intestine of horses due to the digestion of fibrous, it is possible that vitamin B<sub>12</sub> production could be compromised in these animals. Consequently, the observed increase in MCV values is mostly related to a low fiber diet than to the investigated bacteria.

## Conclusion

The contact of horses with *Rickettsia* spp., Anaplasmatocaeae, and *Borrelia* spp. (s.l.) strongly suggests that Campo Grande, Midwest region of Brazil, is an enzootic area for tick-borne zoonotic bacteria. The absence of molecular positivity in all ticks collected indicates that horses do not present persistent bacteremia, suggesting that other mammal species in the studied area may play a role as amplifier

hosts for these zoonotic agents. Horses in the studied area may play a role as a sentinel host for tick-borne zoonotic bacteria. Additionally, monocytopenia with lymphopenia found in positive horses by both *Borrelia* spp. and Anaplasmatocaeae can impair the health of horses.

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**Data availability** The datasets analyzed during the current study are available from the corresponding author on request.

## Declarations

**Ethical approval** All methods were carried out in accordance with the guidelines and regulations of the National Council for Animal Experimentation (CONCEA). The procedures with the horses were approved by the Animal Use Ethics Committee (CEUA) of the Catholic University Dom Bosco, registration number 020/2017.

**Conflict of interest** The authors declare no competing interests.



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