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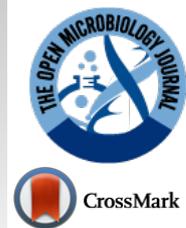
RESEARCH ARTICLE

Equine Granulocytic Anaplasmosis, A Neglected Disease: Risk Factors Associated with Prevalence of Antibodies in Equines

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Abstract:

Background:

Anaplasma phagocytophilum, a tick-borne bacterium that causes granulocytic anaplasmosis, is a neglected pathogen in Brazil, and is diagnosed in several species of domestic and wild animals as well as in humans.

Objective:

This study aimed to investigate the prevalence of anti-*A. phagocytophilum* antibodies in Equidae from the state of Rio de Janeiro and to identify possible risk factors for infection.

Materials and Methods:

A total of 612 blood samples were collected from horses from 15 municipalities within the state. Moreover, an epidemiological questionnaire was administered to evaluate aspects related to seroreaction, taking into account the spatial distribution (properties, municipalities, and mesoregions), management practices, signs of disease, and the individual state of the animals. For the diagnosis, indirect immunofluorescence was performed.

Results:

In the present study, 124 (20.26%), out of a total of 612, animals with anti-*A. phagocytophilum* IgG antibodies at titers of 1:80 were detected. Multivariate logistic regression analysis showed that the presence of the infection at the property (P <0.0001) and the origin (P = 0.0095) of the horse were the true risk factors for infection in the state of Rio de Janeiro.

Conclusion:

This allows to infer that the bacterium is distributed in all mesoregions of the state of Rio de Janeiro and that animals from other states can introduce the infection and make a property a focus of disease; it can also be inferred that these properties are important in the maintenance of the disease and the permanence of bacteria circulating in horses. It is also noteworthy that this was the first identification of mules as hosts of *A. phagocytophilum* infection.

Keywords: Granulocytic anaplasmosis, Hemobacteria, Horses, Mules, IFAT, Anaplasma phagocytophilum.

1. INTRODUCTION

Anaplasma phagocytophilum was reclassified as a gram-negative bacterium of granulocytic cells, including neutrophils

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and eosinophils, from various animal species, including humans, and belonging to the order Rickettsiales and family Anaplasmataceae [1]. This microorganism was identified as the cause of ruminant tick fever in 1940, and as the agent of equine granulocytic anaplasmosis (EGA) in 1969, canine granulocytic anaplasmosis (CGA) in 1982, and in the 1990s, it was found responsible for human granulocytic anaplasmosis (HGA), a

self-limiting feverish illness that can lead to the death of immunosuppressed people [2].

The prevalence of granulocytic anaplasmosis is related to population and tick species, beyond host density and susceptibility, as well as to reservoir species and still the circulating *A. phagocytophilum* strains within the geographical area [3]. It is estimated that 50% of horses in endemic areas are seropositive for *A. phagocytophilum* and recover spontaneously [4]; in some studies, only 5-10% of horses progress clinically to EGA [5]. Clinical signs of anaplasmosis are generally nonspecific, such as high and intermittent fever in the first two days, anorexia, weight loss, lethargy, anemia and edema of the limbs [6], as well as depression, petechiae in the ocular mucosa and oral, jaundice, ataxia and reluctance in movement in the most severe cases [7].

The diagnosis based on clinical signs is not definitive; however, it is in the acute phase, lasting seven to 14 days, where symptoms can be more easily observed [8]. In laboratory diagnosis by blood smear, the presence of an inclusion corpuscle is observed in 1 to 6% of neutrophils after 48 to 72 hours of infection and may persist for up to 15 days after infection. These corpuscles are also observed in equine eosinophils [8 - 11]. The visualization of morulae in the granulocytes is specific for the diagnosis, but the low sensitivity due to the short time of their presence during the disease can result in false negative diagnosis [12]. The most commonly used tests for the diagnosis of EGA are serological tests, including IFAT, followed by ELISA and western immunoblotting [13].

The aim of the present research was to verify the prevalence of anti-*A. phagocytophilum* antibodies in Equidae from Rio de Janeiro state, Brazil, and identify possible risk factors for infection, taking into account geographic distribution, the management practices of the properties, and factors related to individual disease cases to contribute to the surveillance of this agent in the country and worldwide.

2. MATERIALS AND METHODS

Blood of 612 equines was collected from the jugular vein in serum tubes and maintained at +4°C. All sample procedures were performed according to the routine of the Municipal Institute Jorge Veitsman in the use of animals. The samples were centrifuged at 4.000 rpm for 10 min, and the separated sera were stored at −20°C. The sample size was selected based on the total number of 121,594 Equidae reported in the state [14], an expected prevalence of 17.03% and an acceptable prevalence of up to 25%. Of these sera, anti-*A. phagocytophilum* antibodies of the IgG class were searched through IFAT using the IFA Equine Antibody-Fuller Laboratories© kit, following the manufacturer's instructions, with the aid of a Zeiss immunofluorescence microscope (AXIOSKOP 40). Sera that reacted at a dilution of 1:80 were considered positive samples.

In all properties where the samples were collected, an epidemiological questionnaire was administered, giving preference to the veterinarians responsible for the herds,

followed by the treaters or trainers and finally, the owners. To facilitate data organization, the inquiry was divided into four parts according to the classification of its variables, such as: (1) "geographic distribution" in mesoregions, municipalities and sample collection properties; (2) "management" in relation to the practices adopted in the properties, such as the breeding system, production system, water supply, quality of management, type of food, food supplementation, mineral supplementation, regular veterinary assistance, vaccination, hygienic and sanitary conditions, receipt of animals for breeding, reproductive management and acaricide application; (3) "signs of infection" based on questions regarding factors related to the disease, such as history of anemia in the property, presence of dogs, association with breeding of small ruminants, association with cattle breeding, presence of wild animals, commercialization of Equidae, frequent trips and participation in events and/or gatherings; (4) "individualized inquiry" with information from each animal, namely, gender, age range, equine origin, equestrian activity and individual cases of anemia.

From the seroreactivity of the animals and the data collected in epidemiological records, risk factors were evaluated. To verify the relative risks (Rr) relating to the EGA and the various variables, the non-parametric chi-square test and the Fisher exact test were used to determine the odds ratio. To determine the real risk factors when considering multiple factors, the logistic regression was calculated with a 95% confidence interval using the SAS program [15].

3. RESULTS

A total of 612 horses were analyzed in 33 rural properties of 15 municipalities distributed in the six mesoregions of Rio de Janeiro state. Out of these, 124 (20.3%) were seroreactive for anti-*A. phagocytophilum* antibodies. All mesoregions analyzed were positive (100%) for infection. No infection was found in only five (15.1%) properties (Sítio Cajueiro -3%, Central S Francisco -1.6%, Sítio Alto Limoeiro -0.8%, Sítio S Joao Batista -0.3% and Rancho Tropa de Elite -0.3%) making up 6% of the total animals tested, and five other properties (PEC-PM -1,3%, Vale do Marmelo -2.8%, Haras Verde e Preto -2.6%, Rancho dos Amigos -1.3% and Haras Olympo -1.3%) showed significant differences (P<0.0001) in the prevalence of antibodies in the blood (Table **1**).

3.1. Geographic Distribution

Out of the six mesoregions analyzed, only the Northwest was characterized not as a risk factor but rather as a protective factor for AGE (Table **1**). Regarding the municipalities, seven of them (Rio de Janeiro, Friburgo, Teresópolis, Rio Bonito, Campos, Barra do Pirai and Paulo de Frontin) were considered at risk; however, the municipality of Vassouras (3.8%) presented protective characteristics for the infection, and among all the investigated municipalities, São Francisco do Itabapoana was the only municipality where anti-*A. phagocytophilum* antibodies were not detected in the 10 (1.6%) investigated animals (Table **1**). Out of the 33 properties, only one (PEC-PM -6.7%) showed protective characteristics against *A. phagocytophilum* infection (Table **1**).

Table 1. Frequency of anti-*Anaplasma phagocytophilum* antibodies in equine serum evaluated by RIFI in the State of Rio de Janeiro. Brazil.

MESOREGIONS	seroreaction		TOTAL(%)	MUNICIPALITIES	seroreaction		TOTAL(%)	PROPERTIES	seroreaction		TOTAL(%)
	Positive (%)	Negative (%)			Positive(%)	Negative(%)			Positive(%)	Negative(%)	
Metropolitan	16 (2.6)[a]	83 (13.6)	99 (16.2)	Rio de Janeiro	11 (1.8)[a]	80 (13.1)	91 (14.9)	**PEC- PM[3]**	8 (1.3)[a]	33 (5.4)	41 (6.7)
								RPMont	3 (0.5)[c]	47 (7.7)	50 (8.2)
				Tanguá	5 (0.8)[c]	3 (0.5)	8 (1.3)	GM Treinamentos	5 (0.8)[bc]	3 (0.5)	8 (1.3)
Mountain	33 (5.4)[a]	118 (19.3)	151 (24.7)	Friburgo	16 (2.6)[a]	35 (5.7)	51 (8.3)	Vale do Marmelo	17 (2.8)[a]	83 (13.6)	100 (16.4)
				Teresópolis	17 (2.8)[a]	83 (13.6)	100 (16.3)	Haras Verde e Preto	16 (2.6)[a]	35 (5.7)	51 (8.3)
Lakes	16 (2.6)[a]	84 (13.7)	100 (16.3)	Rio Bonito	15 (2.5)[a]	62 (10.0)	77 (12.6)	Haras Toca do Ururau	1 (0.2)[c]	9 (1.5)	10 (1.6)
								Criatório JGO	4 (0.6)[c]	26 (4.2)	30 (4.9)
								Rancho dos Amigos	8 (1.3)[a]	7 (1.2)	15 (2.5)
								Haras Bacaxá	2 (0.3)[c]	20 (3.3)	22 (3.6)
				Silva Jardim	1 (0.2)[c]	22 (3.6)	23 (3.8)	Haras Juturnaíba	1 (0.2)[c]	22 (3.6)	23 (3.8)
North	14 (2.3)[a]	81 (13.2)	95 (15.5)	Campos	12 (1.9)[a]	53 (8.7)	65 (10.6)	Hvet-UENF	4 (0.6)[c]	7 (1.2)	11 (1.8)
								Rancho Elba	4 (0.6)[c]	17 (2.8)	21 (3.4)
								Sítio Cajueiro	0 (0.0)[c]	18 (2.9)	18 (3.0)
								Curral -UENF	4 (0.6)[c]	11 (1.8)	15 (2.5)
				S. F[co.] do Itabapoana	0 (0.0)[c]	10 (1.6)	10 (1.6)	Central S Francisco	0 (0.0)[c]	10 (1.6)	10 (1.6)
				Cardoso Moreira	2 (0.3)[c]	18 (2.9)	20 (3.3)	Sítio Trapiche	3 (0.5)[c]	18 (2.9)	21 (3.4)
Northwest[1]	9 (1.5)[b]	49 (8.0)	58 (9.5)	Itaperuna	5 (0.8)[c]	27 (4.4)	32 (5.2)	Marambaia	1 (0.2)[c]	9 (1.5)	10 (1.6)
								Sítio Água Santa	3 (0.5)[c]	13 (2.1)	16 (2.6)
								Sítio Alto Limoeiro	0 (0.0)[c]	5 (0.8)	5 (0.8)
				Natividade	4 (0.6)[c]	22 (3.6)	26 (4.2)	Sítio dos Palmares	3 (0.5)[c]	13 (2.1)	16 (2.6)
								Sítio Pau-Ferro	1 (0.2)[c]	9 (1.5)	10 (1.6)
South Fluminense	36 (5.9)[a]	73 (11.9)	109 (17.8)	**Vassouras[2]**	8 (1.3)[b]	15 (2.5)	23 (3.8)	Haras Olympo	8 (1.3)[a]	15 (2.4)	23 (3.8)
				Valença	1 (0.2)[c]	11 (1.8)	12 (2.0)	Rancho da Cachoeira	1 (0.2)[c]	11 (1.8)	12 (2.0)
				Barra do Pirai	15 (2.5)[a]	24 (3.9)	39 (6.4)	Jorge Lima Carvalho	2 (0.4)[c]	6 (1.0)	8 (1.3)
								Sitio S Joao Batista	0 (0.0)[c]	2 (0.3)	2 (0.3)
								Rancho Tropa de Elite	0 (0.0)[c]	2 (0.3)	2 (0.3)
								Sergio Righ	1 (0.2)[c]	4 (0.6)	5 (0.8)
								Fazenda Aterrado	5 (0.8)[bc]	5 (0.8)	10 (1.6)
								Haras FRF	2 (0.3)[c]	3 (0.5)	5 (0.8)
								Haras Ponto do Retiro	5 (0.8)[b]	2 (0.3)	7 (1.2)
				Paulo de Frontin	12 (1.9)[a]	23 (3.8)	35 (5.7%)	Herdade das Palmas	5 (0.8)[b]	6 (1.0)	11 (1.8)
								Haras Pedro Valente	3 (0.5)[c]	16 (2.6)	19 (3.1)
								Haras Ana C. Batista	4 (0.6)[c]	1 (0.2)	5 (0.8)
TOTAL	**124 (20.3%)**	**488 (79.7%)**	**612 (100)**	**TOTAL**	**124 (20.3)**	**488 (79.7)**	**612 (100)**	**TOTAL**	**124 (20.3)**	**488 (79.7)**	**612 (100)**

Equal letters in the same column do not differ statistically with a 95% confidence interval. [1]P= 0.0130 [χ²=16.1515 (95% CI: 1.1381-5.7780)] e (*Rr*<**0.001).**[2]P=0.002 [χ²=39.3755 (95% CI: 3.8338-13.8467)] e (*Rr*<**0.001).**[3]P<0.0001 [χ²=87.8745 (95% CI: 1.5349-86.4092)] e (*Rr*<**0.001).**

3.2. *Management*

Out of the 13 analyzed parameters related to management, four presented statistical significance regarding the prevalence of anti-*A. phagocytophilum* antibodies in equine sera, namely, food supplementation (P=0.0001), hygienic conditions (P=0.0136), reproductive management (P=0.0038), and application of acaricides (P=0.0428) (Table **2**).

Animals receiving food supplementation during the dry

season were considered less susceptible than animals receiving no supplementation (Table **2**).

It was observed that 83.5% of equines received regular veterinary assistance, and 67.8% were vaccinated for at least six types of diseases, specifically, rabies, equine influenza, tetanus, encephalomyelitis, equine herpes virus, and leptospirosis (Table **2**).

Considering the hygienic and sanitary conditions of the facilities where the Equidae were reared, it was observed that animals reared in good (44.9%) and regular (47.1%) conditions are more susceptible to *A. phagocytophilum* infections than animals that live under excellent (3.9%) and poor (4.1%) conditions (Table **2**).

Properties that do not use any kind of reproductive technology are more likely to present seroreactive animals. The use of acaricides has been found to be a protective factor; untreated animals are twice as likely to contract *A. phagocytophilum* infection (Table **2**).

3.3. Evidence of Infection

Regarding the presence of factors that may favor the infection, no significant differences were observed among the eight evaluated parameters: Anemia history on the property, presence of dogs, association with other creations, cattle breeding association, presence of wild animals, commercialization of equidae, frequent trips and participation events and / or gatherings (Table **3**).

Table 2. Frequency of anti-*Anaplasma phagocytophilum* antibodies in equine serum according to management-related variables applied to properties of Rio de Janeiro state, Brazil.

PARAMETERS	FREQUENCIES		TOTAL [n. (%)]	STATISTIC
	Sororeactive [n. (%)]	Negatives [n. (%)]		
Breeding system				
Extensive	19 (3.1)	53 (8.7)	72 (11.8)	P=0.3625 [χ^2= 2.0295 (95% CI: 0.3816-0.4862)].
Semi-intensive	68 (11.1)	290 (47.4)	358 (58.5)	
Intensive	37 (6.1)	145 (23.7)	182 (29.7)	
Production system				
Familiar	22 (3.6)	70 (11.4)	92 (15.1)	P=0.3884 [χ^2=1.8917 (95% CI:0.3929-0.9731)].
Pre-family	35 (5.7)	122 (19.9)	157 (25.6)	
Business	67 (10.9)	296 (48.4)	363 (59.3)	
Water supply				
Own source	115 (18.8)	449 (73.3)	564 (92.2)	P=0.07861 [χ^2=0.0736 (95% CI:0.7863-0.9328)]
Public network	9 (1.5)	39 (6.4)	48 (7.8)	
Quality of management				
Excellent	7 (1.1)	17 (2.8)	24 (3.9)	P=0.2319 [χ^2=4.2898 (95% CI:0.2699-0.7047)]
Good	53 (8.7)	222 (36.3)	275 (44.9)	
Regular	52 (8.5)	222 (36.3)	274 (44.8)	
Bad	12 (2.0)	27 (4.3)	39 (6.4)	
Feed Type				
Pasture Only	13 (2.1)	41 (6.7)	54 (8.8)	P=0.4523 [χ^2=1.5868 (95% CI: 0.4510-0.7325)]
Pasture and concentrate	39 (6.4)	181 (29.6)	220 (36.0)	
Hay and concentrate	72 (11.8)	266 (43.5)	338 (55.2)	
Food Supplementation				
Hay [(A)]	15 (2.4)	115 (18.8)	130 (21.2)	**P=0.0001 [χ^2=22.7969 (95% CI: <0.0001-0.0309)].**
Fodder [(A)]	27 (4.4)	70 (11.4)	97 (15.8)	
Concentrate [(A)]	9 (1.5)	82 (13.4)	91 (14.9)	
Others [(A)]	20 (3.3)	40 (6.5)	60 (9.8)	
None [(B)]	53 (8.7)	181 (29.6)	234 (38.3)	
Mineral supplementation				
None	7 (1.2)	27 (4.4)	34 (5.6)	P=0.5626 [χ^2=2.9715 (95% CI:0.2675-0.4516)].
Common salt	1 (0.2)	9 (1.5)	10 (1.6)	
Common Salt and Mineral Salt	50 (8.2)	223 (36.4)	273 (44.6)	
Mineral salt	66 (10.8)	229 (37.4)	295 (48.2)	

(Table 2) contd.....

PARAMETERS	FREQUENCIES		TOTAL [n. (%)]	STATISTIC
	Sororeactive [n. (%)]	Negatives [n. (%)]		
Regular Veterinary Assistance				
Yes	104 (17.0)	407 (66.5)	511 (83.5)	P=0.9000 [χ^2=0.0158 (95% CI:0.5111-0.5964)]
No	20 (3.3)	81 (13.2)	101 (16.5)	
Vaccination				
Anti-rabies only	26 (4.2)	96 (15.7)	122 (19.9)	P=0.0928 [χ^2=6.4228 (95% CI:0.0021-0.9631)]
Anti-rabies plus another	1 (0.2)	14 (2.3)	15 (2.5)	
Anti-rabies plus two others	6 (1.0)	54 (8.8)	60 (9.8)	
All vaccines	91 (14.9)	324 (52.9)	415 (67.8)	
Hygienic and sanitary conditions				
Excellent (A)	7 (1.1)	17 (2.8)	24 (3.9)	**P=0.0136 [χ^2 10.6801 (95% CI: 0.0288-0.4919)]**
Good (B)	53 (8.7)	222 (36.3)	275 (44.9)	
Regular (B)	53 (8.7)	235 (38.4)	288 (47.1)	
Bad (A)	11 (1.8)	14 (2.3)	25 (4.1)	
Receipt of animals for breeding				
No	64 (10.5)	221 (36.1)	285 (46.6)	P=0.3624 [χ^2= 2.0300 (95% CI: 0.3571-0.4084)]
Rarely	31 (5.1)	151 (24.7)	182 (29.7)	
Often	29 (4.7)	116 (18.9)	145 (23.7)	
Reproductive management				
Natural mating (Nm) (A)	24 (3.9)	155 (25.3)	179 (29.2)	**P=0.0038 [χ^2=17.4220 (95% CI: 0.0021-0.0046)]**
Artificial insemination (Ai) (A)	11 (1.8)	45 (7.3)	56 (9.2)	
(Nm) + (Ai) (A)	25 (4.1)	76 (12.4)	101 (16.5)	
Embryo transference (Et) (B)	0 (0)	10 (1.6)	10 (1.6)	
(Nm) + (Ai) + (Et) (A)	22 (3.6)	41 (6.7)	63 (10.3)	
Any (C)	42 (6.9)	161 (26.3)	203 (33.2)	
Acaricide application				**P=0.0428 [χ^2=4.1045 (95% CI: 0.0429-0.0605)]**
Yes	101 (16.5)	431 (70.4)	532 (86.9)	**P=0.0428 [Rr= 0.0161 (95%CI: 0.0335-0.9826)]**
No	23 (3.8)	57 (9.3)	80 (13.1)	**P=0.0446 [Or=0.5808 (95% CI:0.3417-0.9871)]**

Equal superscript letters in the same items of the respective parameters do not differ statistically.

Table 3. Frequency of anti-Anaplasma phagocytophilum antibodies in equine serum according to variables related to signs of infection in the properties of Rio de Janeiro state, Brazil.

PARAMETERS	FREQUENCIES		TOTAL [n. (%)]	STATISTIC
	Sororeactive [n. (%)]	Negatives [n. (%)]		
Anemia history on the property				
Yes	110 (18.0)	411 (67.2)	521 (85.1)	P=0.2097 [χ^2=1.5736 (95% CI:0.2101-0.2657)]
No	14 (2.3)	77 (12.5)	91 (14.9)	
Presence of dogs				
Yes	124 (20.3)	485 (79.2)	609 (99.5)	P=0.3814 [χ^2=0.7661 (95% CI:0.3818-0.8718)]
No	0 (0)	3 (0.5)	3 (0.5)	
Association with other creations				
Yes	61 (10.0)	212 (34.6)	273 (44.6)	P=0.2500 [χ^2=1.3234 (95% CI:0.2504-0.2941)]
No	63 (10.3)	276 (45.1)	339 (55.4)	
Cattle breeding association				
Yes	40 (6.6)	128 (20.9)	168 (27.4)	P=0.1792 [χ^2=1.8044 (95% CI:0.1795-0.2185)].
No	84 (13.7)	360 (58.8)	444 (72.6)	
Presence of wild animals				
Yes	117 (19.1)	468 (76.4)	585 (95.6)	P=0.4539 [χ^2=0.5610 (95% CI:0.4542-0.6142)]
No	7 (1.2)	20 (3.3)	27 (4.4)	
Commercialization of equidae				
Yes	80 (13.1)	348 (55.9)	428 (69.9)	P=0.1406 [χ^2=2.1715 (95% CI:0.1409-0.1726)]
No	44 (7.2)	140 (22.3)	184 (30.1)	

(Table 3) contd.....

PARAMETERS	FREQUENCIES		TOTAL [n. (%)]	STATISTIC
	Sororeactive [n. (%)]	Negatives [n. (%)]		
Frequent trips				
Yes	102 (16.7)	402 (65.7)	504 (82.4)	P=0.9752 [χ^2=0.0010 (95% CI:0.9753-1.0)]
No	22 (3.6)	86 (14.1)	108 (17.6)	
Participation events and / or gatherings				P=0.1680 [χ^2=1.9004 (95% CI:0.1684-0.2270)]
Yes	108 (17.7)	445 (72.7)	553 (90.4)	
No	16 (2.6)	43 (7.0)	59 (9.6)	

3.4. Individualized Inquiry

Considering the Equidae individually, based on the five parameters evaluated, the origin of the Equidae and the individual case reports of anemia were the ones that presented significant differences (P=0.0098) in their variables regarding the prevalence of anti-*A. phagocytophilum* antibodies (Table **4**). Animals born on the property and those from another state showed significant differences (P=0.0005) in *A. phagocytophilum* infection compared with animals of other origins (Table **4**). Out of the 612 horses that were evaluated, 101 animals with anemia and 511 without anemia were observed. A lack of anemia is considered a protective factor for the presence of anti-*A. phagocytophilum* antibodies (Table **4**).

In the present research, it was found that race and species influence *A. phagocytophilum* infection in Equidae in the state of Rio de Janeiro, although no seroreactive Pampa and Paint Horse animals were observed (Table **5**).

Table 4. Frequency of anti-*Anaplasma phagocytophilum* antibodies in equine serum according to variables related to the individualized survey of animals from Rio de Janeiro state, Brazil.

PARAMETERS	FREQUENCIES		TOTAL [n. (%)]	STATISTIC
	Sororeactive [n. (%)]	Negatives [n. (%)]		
Gender				
Male	55 (9.0)	257 (42.0)	312 (51.0)	P=0.0984 [χ^2= 2.7316 (95% CI:0.987-0.1206)]
Female	69 (11.3)	231 (37.7)	300 (49.0)	
Age range				
Young Foal (0 to 24 months)	18 (2.9)	75 (12.2)	93 (15.3)	P=0.8586 [χ^2=0.7616 (95% CI:0.2208-0.6385)]
Mature Foal (25 to 48 months)	31 (5.2)	135 (22.1)	166 (27.0)	
Adults (49 months to 14 years)	65 (10.6)	235 (38.4)	300 (49.2)	
Seniors (over 15 years)	10 (1.6)	43 (7.0)	53 (8.5)	
Origin of equines				
Own property [A]	34 (5.5)	134 (21.9)	168 (27.5)	**P=0.0005 [χ^2=24.0802 (95% CI: 0.0762-0.0917)**
Neighborhood [B]	22 (3.6)	40 (6.5)	62 (10.1)	
Own municipality [C]	13 (2.1)	102 (16.7)	115 (18.8)	
Other municipality [C]	5 (0.8)	19 (3.1)	24 (3.9)	
Other state [A]	45 (7.3)	188 (30.7)	233 (38.1)	
Other country [C]	5 (0.8)	5 (0.8)	10 (1.6)	
Equestrian activity				
Rural equestrianism	23 (3.7)	93 (15.2)	116 (19.0)	P= 0.2022 [χ^2=12.2020 (95% CI:0.1982-0.6562)]
Job	12 (2.0)	31 (5.1)	43 (7.0)	
Reproduction	14 (2.3)	84 (13.7)	98 (16.0)	
Tour	12 (2.0)	45 (7.3)	57 (9.3)	
Classic equestrianism	17 (2.8)	35 (5.7)	52 (8.5)	
Running	33 (5.4)	118 (19.3)	151 (24.7)	
Policing	13 (2.1)	82 (13.4)	95 (15.5)	
Individual cases of anemia				**P=0.0098 [χ^2=6.6742(95% CI:0.0098-0.0144)].** P=0.0045 [**Rr=0.0142(95% CI:0.0088-0.9957)**]. P=0.1415 [**Or=0.7315**(95% CI:0.4821-1.1098)].
Yes	30 (4.9)	71 (11.6)	101 (16.5)	
No	94 (15.4)	417 (68.1)	511 (83.5)	

Equal superscript letters in the same items of the respective parameters do not differ statistically.

Table 5. Frequency of anti-*Anaplasma phagocytophilum* antibodies infection in relation to equine races in Rio de Janeiro state, Brazil.

PARAMETERS	SEROREACTION		TOTAL	STATISTIC
	Positive n. (%)	Negative n. (%)		
Equines				
Mangalarga[(A)]	26 (4.25)	112 (18.30)	138 (22.55)	
Campolina[(A)]	8 (1.31)	44 (7.19)	52 (8.50)	
English thoroughbred [(A)]	35 (5.72)	120 (19.61)	155 (25.33)	
Arabic [(B)]	2 (0.33)	5 (0.82)	7 (1.14)	
Quarter Horse [(A)]	15 (2.45)	44 (7.19)	59 (9.64)	
Mixed race [(A)]	9 (1.47)	33 (5.39)	42 (6.86)	
English thoroughbred + Brazilian Equestrian [(B)]	3 (0.49)	16 (2.61)	19 (3.10)	
Creole + Brazilian Equestrian [(B)]	2 (0.33)	49 (8.01)	51 (8.33)	**P=0.0401**
Brazilian Equestrian [(A)]	11 (1.80)	28 (4.58)	39 (6.37)	$[\chi^2=24.4760(95\%CI:0.4346\text{-}0.6105)]$
Andalusian [(B)]	1 (0.16)	1 (0.16)	2 (0.33)	
Pampa[(B)]	0 (0.00)	6 (0.98)	6 (0.98)	
Paint Horse[(B)]	0 (0.00)	7 (1.14)	7 (1.14)	
Lusitano[(B)]	3 (0.49)	2 (0.33)	5 (0.82)	
Holstainer[(B)]	7 (1.14)	14 (2.29)	21 (3.43)	
Mules				
Donkey[(B)]	2 (0.33)	7 (1.14)	9 (1.47)	
TOTAL	**124 (20.3)**	**488 (79.7)**	**612 (100)**	

Superscript equal letters in parentheses in the column do not differ statistically.

3.5. *Multivariate logistic Regression*

Taking into account the prevalence of anti-*A. phagocytophilum* antibody seroreactivity and the negative correlations of the influence of one factor on others, it was observed that property and origin (Table **6**) are the true risk factors for infection.

Table 6. Risk factors associated with the frequency of seroreaction against *A. phagocytophilum* in equidae in the state of Rio de Janeiro estimated by multivariate logistic regression method based on the analysis of data collected from an epidemiological record.

VARIABLES	STATISTIC		
	Number	Chi-Square	P Value[1]
Properties	31	87,8745	< 0,0001
Origin	6	16,9456	0,0095

[1]Values with 95% confidence interval.

4. DISCUSSION

For the diagnosis of *A. phagocytophilum* infections, the IFAT-paired serology method was chosen because it is considered the "gold standard" by the World Health Organization in the search for anti-*A. phagocytophilum* antibodies [16], in addition to being the routine technique for serological monitoring of infection in reference laboratories when the objective is bacterium tracking in communities [17]. Moreover, the diagnosis of the bacterium in the peripheral blood is limited, even in acute infections, due to the short duration of the bacteremia, which makes the isolation in smears or molecular biology difficult. In the chronic phase, all these procedures are even more difficult due to the possible elimination of the bacterium from the organism over time [18, 4, 19].

The observed prevalence of 20.3%, distributed throughout the state of Rio de Janeiro, without observation of clinical signs of the disease, suggests that EGA is being underdiagnosed or confused with other diseases, such as piroplasmosis, equine infectious anemia, rhabdomyolysis, and leptospirosis. The prevalence observed in this study (22.3%) was observed by other authors [5] in Denmark, who also did not observe clinical signs of the disease. In addition, it is known that in a seroreactive population, the infection has been occurring for more than three weeks, and antibodies persist for a long period, therefore blood samples can be collected more than three months after infection initiation [20, 12, 5, 16].

Both breeding and production systems were not determinant in *A. phagocytophilum* infection in horses in this research, although another study [21] observed in horses in Sweden that grazing time is correlated with positive seroreactivity to anti-*A. phagocytophilum* antibodies and that the extensive and semi-intensive systems presented higher seroreactivity than confined groups.

The quality of management used in the rearing and the origin of the water the Equidae received did not influence the prevalence of *A. phagocytophilum* infection. The result relating to water was expected because this agent is not water-borne; however, unlike in this study, another study [22] observed that horses reared in poorly managed conditions are more susceptible to disease.

It is known that some nutritional deficiencies and mineral imbalances in horses may lead to anemia, immunodepression, and concomitant diseases [23], but the types of food or mineral

supplementation provided to animals were not determining factors in the infection by *A. phagocytophilum* in the equines of this research. The animals not supplemented during the dry season presented the highest rates of seropositivity among the groups surveyed, which may be associated with a malnutrition condition that may favor immunodepression and acquisition of opportunistic infectious agents, according to another study [24].

The presence of permanent veterinary care is a favorable factor for the prophylaxis and control of several diseases in farm animals [23]; however, the presence of veterinarians on the property and the number of vaccines applied were not determining factors in the control of *A. phagocytophilum* infection in animals.

Seroreaction to anti-*A. phagocytophilum* antibodies was detected in 23.7% of Equidae on properties that frequently received breeding animals, with no significant difference related to this practice (P=0.3624), and a significant difference (P=0.0038) in seroreaction prevalence was observed in Equidae (33.2%) that did not receive any type of reproductive management, a result also observed in another study [5].

In Brazil, especially in the Southeast region, the records found of tick-borne diseases are for borreliosis and spotted fever, which have a mortality of up to 30% in humans [25]. These same authors mention that these diseases have worldwide relevance, especially in countries of the northern hemisphere. In this research, it was observed that most horses that were analyzed received tick treatment (86.9%), and a significantly higher prevalence (P=0.0428) of seroreactivity was observed within the untreated group than within those treated with ectoparasiticides. In addition, tick treatment was considered a protective factor, and it was observed that untreated animals are twice as likely as treated animals to contract EGA. Another study [24] observed that horses from various regions of Central America that are diagnosed as positive for anti-*A. phagocytophilum* antibodies are parasitized by the same tick species, *Amblyomma sculptum* (synonymy *A. cajennense*) and *A. nitens*, which are also reported to parasitize the Equidae from the Southeast region of Brazil. The maintenance of the *A. phagocytophilum* biological cycle is known to depend on tick density and the tick species involved [26]. The 16.5% prevalence of seroreactivity in treated animals in relation to the total analyzed may suggest incorrect antiparasitic control contributing to tick resistance and vector permanence in the herd [27, 28]. A collective history of anemia is not a useful parameter for suspected EGA in the herds, as the difference in the prevalence of seroreaction in anemic compared with nonanemic herds was not statistically significant (P=0.2097). Similar results were also observed in another study [4] when evaluating horses that were experimentally infected with *A. phagocytophilum* for four months. All animals developed acute clinical signs such as fever, inappetence, ataxia, and hind limb edema; however, anemia was not a problem, generally speaking. However, this variable was characterized as a risk factor when individually assessed, contrary to the observation for the history of anemia in the properties evaluated in the present research.

Dogs carrying *A. phagocytophilum* have already been detected in Rio de Janeiro [29]; in addition, the importance of dogs as a source of the infection for the vectors is known. However, in this research, there was no characterization of the presence of this animal species on equine farms as significant for *A. phagocytophilum* infection (P=0.3814).

As for conviviality with small ruminants, the findings of this research did not recognize this factor as significant for the prevalence of seroreaction against *A. phagocytophilum* (P=0.2500); however, other authors, when studying the prevalence of this pathogen in properties of Italy, observed that horses raised in association with small ruminants present significantly higher rates of infection [22]. Specifically to the presence of cattle, although 27.4% of the equines examined in this study live with these animals, this variable was not significantly (P=0.1792) associated with the positivity index, suggesting that the cattle were not relevant as hosts or reservoirs of *A. phagocytophilum* in the investigated properties. Another study observed the presence of *Rhipicephalus microplus* and non-infection by Ixodes ticks in cattle when integrated or alternating grazing between these and horses [24], reporting that integrated breeding of these two species does not pose a risk to Equidae in terms of the prevalence of infection by *A. phagocytophilum*.

In the epidemiological survey in this research, the presence of wild animals was observed in 95.6% of the investigated properties, with 19.1% of Equidae being seroreactive (Table **3**). According to other authors [30], even in regions where the prevalence of EGA is low, tick monitoring, prevention, and control programs should be established, mainly in areas near forests where there are several species of the genus *Ixodes*, which infest the wild fauna. According to other studies, ticks of genera *Ixodes* and *Amblyomma* are also commonly found in wild rodents, lagomorphs, cervids, marsupials, carnivores, reptiles, and birds [31]. However, the presence of wild animals was not considered a significant factor (P=0.4539) for the presence of *A. phagocytophilum* infection in the Equidae of Rio de Janeiro state.

Although commercialization, frequent travel, and participation in events and/or gatherings are stress factors for animals and can contribute to immunodepression and consequently infectious diseases [21, 32], the respective prevalences of 13.1%, 16.7%, and 17.7% of seroreactivity associated with these variables in this research indicated that these factors are not significant in *A. phagocytophilum* infection (P=0.1406, P=0.9752, P=0.1680, respectively); however, these activities were considered a risk factor for disease in horses [21].

Out of the 612 Equidae investigated from the state of Rio, there was no significant difference in seroreactivity for *A. phagocytophilum* between sexes (P=0.0984), corroborating the findings of authors of a study [33], who also did not observe differences in infection according to the sex of the animal in 733 horses studied in the region of Minnesota and Wisconsin (USA).

By stratifying the examined equine herd according to age group, it was found that the highest numbers of seroreactive animals corresponded to foals and adult animals, evidencing

that any individual is subject to reinfection with detectable serological titers, with no statistically significant difference in the prevalence of seroreactivity of the animals (P=0.8586). However, a study found a significant correlation (P≤0.03) between age over 11 years in horses and the presence of anti-*A. phagocytophilum* antibodies [5], and another one concluded that the prevalence increases with age [32]. Thus, age constitutes an important risk factor due to the higher prevalence of tick exposure throughout an animal's life.

Equidae from the property and from other states presented a significantly higher prevalence of seroreaction to *A. phagocytophilum,* when compared to animals from other origins (P=0.0005). This is one of the most important variables in this study because the logistic regression analysis shows the possibility of foci of infection located in properties or microregions, as observed for other diseases, such as equine infectious anemia [34].

Seroreactive animals were found for all equine aptitude categories evaluated in this research. However, no aptitude category was significant for seroreaction against *A. phagocytophilum*, which further raises concern about this zoonosis with possible EGA dispersal because the variety of equestrian activities intensify equine transit, which may favor the spread of the disease [25], including its spread to humans.

In this research, the prevalence of seroreactive animals in properties with a history of anemia was not significant (P=0.2097); however, anemia, as individually assessed in equines, was significantly associated with infection (P=0.0098), and the absence of anemia in animals was a protective factor against infection. In addition, another study stated that *A. phagocytophilum* is responsible for important hematological complications, such as alterations in erythro-poiesis, suppression of erythroid progenitor cell proliferation, erythroid cell apoptosis and reduced iron availability, which may contribute to the development of anemia and cytopenias [35].

Regarding the equine breeds analyzed from this research in the state of Rio de Janeiro, significant differences in seroreactivity for anti-*A. phagocytophilum* antibodies were observed among breeds (P=0.0401). In contrast, other authors did not observe significant differences when evaluating 23 equine breeds in the United States [33]. According to other studies [33, 21], horses used for racing are an important risk factor. Another risk factor to consider is that genetically pure breeds, such as English thoroughbred, Holstainer, and Mangalarga, are more sensitive to tick infections [36]. Although studies in asinines and mules are scarce, a study found 6% seroreactivity (6/100) in asymptomatic asinines tested in Italy [37]. This result, together with the 22.2% (2/9) seroreactivity in the mules surveyed in the state of Rio de Janeiro, corroborates that this animal species also participates in the epidemiological chain of EGA.

Comparing the distribution of the prevalence of seroreaction against *A. phagocytophilum* in the properties and its prevalence according to the origin of the equines and to the other variables where significance was observed in the seroreaction observed in this research, multivariate logistic regression analysis indicated that the distribution of infection on properties (P<0.0001) and the origin (P=0.0095) of Equidae were the real risk factors for *A. phagocytophilum* infection. These results corroborate those in researches conducted in Europe [21, 32].

CONCLUSION

After analyzing the epidemiological factors for the presence of anti-*A. phagocytophilum* antibodies in Equidae from the state of Rio de Janeiro, it can be inferred that EGA is present in the equine herds of the state in an enzootic manner. Although the agent is widely distributed in all mesoregions of the state of Rio de Janeiro, the focus on properties is important in the permanence of the disease in the herds. Thus, attention should be paid to the transit of Equidae, mainly from other states, to prevent the introduction of the etiological agent *A. phagocytophilum* to the property and prevent it from becoming a focus of the disease. This research indicates that mules are also important as hosts or reservoirs, therefore investigations should be developed to better clarify the importance of these animals in the epidemiological chain of EGA.

AUTHOR CONTRIBUTION STATEMENT

Francisco C. R. de Oliveira: Prepared the tables and wrote the main manuscript text.

Marcia F. Rolim: Collected the data and wrote the main manuscript text.

Samira S. M. Gallo: Wrote the main manuscript text.

Célia R. Quirino: Performed data statistics.

Nicole B. Ederli: Wrote the main manuscript text.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The authors confirmed that the study does not have an ethical committee number, because it was a routine exams. They collect blood samples for routine examinations.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

CONFLICT OF INTEREST

ACKNOWLEDGEMENTS

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