Revista Brasileira de Parasitologia Veterinária
Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv

Original Article

Sarcocystis neurona, seroprevalence of antibodies in equines and research of oocystis in opossum in Ilhéus - Itabuna microregion, Bahia, Brazil

Sarcocystis neurona, seroprevalência de anticorpos em equinos e pesquisa de oocistos em gambás na microrregião de Ilhéus - Itabuna, Bahia, Brasil

Samantha Gusmão Pellizzoni[1]; Sônia Carmen Lopo Costa[2]; Raissa Barros Gracie Mery[3]; Jonata Melo Barbieri[4]; Alexandre Dias Munhoz[1]; Aísla Nascimento da Silva[1]; Anaiá da Paixão Sevá[1]; Martín Roberto del Valle Alvarez[5]; George Rêgo Albuquerque[1]*

[1] Programa de Pós-graduação em Ciência Animal, Universidade Estadual de Santa Cruz – UESC, Ilhéus, BA, Brasil
[2] Centro de Controle de Zoonoses, Prefeitura Municipal de Itabuna, Itabuna, BA, Brasil
[3] Médica Veterinária Autônoma, Ilhéus, BA, Brasil
[4] Programa de Pós-graduação em Medicina Veterinária, Escola de Veterinária, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, MG, Brasil
[5] Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz – UESC, Ilhéus, BA, Brasil

How to cite: Pellizzoni SG, Costa SCL, Mery RBG, Barbieri JM, Munhoz AD, Silva AN, et al. *Sarcocystis neurona,* seroprevalence of antibodies in equines and research of oocystis in opossum in Ilhéus - Itabuna microregion, Bahia, Brazil. *Braz J Vet Parasitol* 2021; 30(2): e027720. https://doi.org/10.1590/S1984-29612021054

Abstract

The aims of this study were to determine the seroprevalence of *Sarcocystis neurona* antibodies in equines in the Ilhéus-Itabuna microregion (BA), and identify possible factors associated with infection. The presence of sporocysts/oocysts of *Sarcocystis* spp. was also verified in *Didelphis* spp. A total of 669 serum samples were collected from equines in 56 properties located in 12 municipalities in the region. Indirect fluorescent antibody test was performed with slides containing merozoites of the *S. neurona*, using a cut-off titer of 1:80. Occurrence of 7.92% of anti-*S. neurona* antibodies was observed in the sampled equines. The purposes trade and work were significantly associated with the presence of antibodies ($p<0.05$), and being used for the purpose of work (21.6%) was considered a risk factor, while being used for the purpose of trade (3.6%) was a protective factor. A total of 25 *Didelphis* spp. was captured for research on sporocysts/oocysts in stool samples and intestinal scrapings, being all negative. *Didelphis* spp. were all negative for the presence of *Sarcocystis* spp. and this circumstance does not change the fact that seroprevalence of *S. neurona* has been observed in horses raised in the southern Bahia.

Keywords: Equine protozoal myeloencephalitis, *Sarcocystis*, *Didelphis*.

Resumo

O presente estudo foi realizado na microrregião de Ilhéus-Itabuna, Bahia. Os objetivos deste estudo foram determinar a soroprevalência de anticorpos contra *Sarcocystis neurona* em equinos da microrregião Ilhéus-Itabuna (BA) e identificar possíveis fatores associados à infecção. A presença de esporocistos/oocistos de *Sarcocystis* spp. também foi pesquisada em *Didelphis* spp. Foram coletadas 669 amostras de soro de equinos em 56 propriedades localizadas em 12 municípios da região. Foi utilizada a reação de imunofluorescência indireta (RIFI), utilizando-se lâminas confeccionadas com merozoítos de *Sarcocystis neurona*, (cepa SN138) e ponto de corte na diluição de 1:80. A ocorrência de anticorpos anti- *S. neurona* nos equinos amostrados, foi de 7,92%. As finalidades dos animais - comércio e trabalho - apresentaram-se significativas ($p<0.05$), sendo que a finalidade trabalho (21,6%) foi considerada fator de risco, enquanto a finalidade comércio (3,6%) foi considerada fator de proteção. Foram capturados 25 *Didelphis* spp., para pesquisa de esporocistos/oocistos em amostras de fezes e raspado de mucosa intestinal. Todos os *Didelphis* spp. foram negativos para a presença de *Sarcocystis* spp.,

Received November 24, 2020. Accepted May 10, 2021.
*Corresponding author: George Rêgo Albuquerque. E-mail: gralbu@uesc.br

mesmo assim essa circunstância não alterou o fato da ocorrência de *S. neurona* ter sido observada em cavalos criados na mesorregião do sul da Bahia.

Palavras-chave: Mieloencefalite protozoária equina, *Sarcocystis*, *Didelphis*.

Introduction

Brazil has a herd of more than 5 million horses (Lima & Cintra, 2016). It represents the third largest herd in the world and the largest in Latin America, moving approximately R$ 16.15 billion annually and generating around 610 thousand direct jobs and 2.4 million indirect jobs (Lima & Cintra, 2016). Bahia occupy the 3rd position on the national ranking, with 8.6% of the equine herd (IBGE, 2016).

Equine Protozoal Myeloencephalitis (EPM) is a debilitating and progressive neurological disorder that affect equines. Its main etiological agent is *Sarcocystis neurona*, a protozoan belonging to Apicomplexa phylum, Eucoccidiida order and Sarcocystidae family (Dubey et al., 2015). The main clinical sign is incoordination, resulting from decreased proprioception and muscle weakness. There is also neurogenic muscle atrophy and cranial nerve paralysis (Smith, 1994; Dubey & Miller, 1986; Thomassian, 2005; Dubey et al., 2015).

The definitive hosts of *S. neurona* are marsupials of the *Didelphis* genus, *Didelphis virginiana* in North America (Dubey & Lindsay, 1998), and *D. albiventris* in South America (Dubey et al., 2001a). In South America, other species of opossum (*D. aurita* and *D. marsupialis*) may be involved in the transmission of the agent (Silva et al., 2003). A variety of mammals may act as intermediate hosts, including skunks, raccoons, armadillos, cats and sea otters (Dubey et al., 2015).

Horses accidentally become infected when they ingest water and food contaminated with sporocysts eliminated in the feces of the definitive host. After ingestion, sporozoites are released into the small intestine and asexual reproduction begins in the vascular endothelium, from where the parasites, in the form of merozoites, migrate into the bloodstream, cross the blood-brain barrier, and reach the Central Nervous System (MacKay, 2001).

The main risk factors for the emergence of EPM are related to age, geographic proximity to the occurrence of the definitive host, environmental factors (climate and hydrography), food management system, purpose of animal husbandry (sport, reproduction and work) being exposed to greater stress situations, that is, transportation, intense training, participation in races, postpartum and constant activities (Reed & Bayly, 2000; Dubey et al., 2015).

Anti-*S. neurona* antibodies have been found in equines in several countries with seroprevalence ranging from 0% in New Zealand (Vardeleon et al., 2001) to 89.2% in the United States (Bentz et al., 2003). In Brazil, records of equine exposure to the protozoa are scarce, with few studies having verified seroprevalence of *S. neurona* infection in the equine troop, with numbers ranging from 1.6% in Alagoas Northeast Region, (Valença et al., 2019) to 90,00% in Minas Gerais (Hoane et al. 2006).

Equine protozoan myeloencephalitis (EPM) had its first case reported in Brazil in Rio Grande do Sul, by Barros et al. (1986) in a 10-years-old horse. Clinical case reports or studies investigating EPM have been reported in several Brazilian states (Masri et al., 1992; Lins et al., 2008; Faria et al., 2017). Henker et al. (2020) concludes that EPM due to *S. neurona* infection represents an important neurologic disease of horses in Brazil and this disease should be considered as a main differential diagnosis in horses presenting neurologic signs.

Bahia is a state located in the Northeast region of the country. Although the data have not been published, some clinical cases characteristic of EPM have already been diagnosed in the southern mesoregion of the state. Therefore, the aim of this study was to determine seroprevalence and factors associated with *S. neurona* infection in equines in the Ilhéus-Itabuna microregion, Bahia, Brazil. To identify the dynamics of the disease in this population, *Sarcocystis* infection in the definitive hosts (opossums) of the region was also evaluated.

Material and Methods

Study population and sample collection

The study was carried out in the South Bahia mesoregion, Ilhéus-Itabuna microregion, comprising 12 municipalities with a high number of horse farms, these being: Itabuna, Itapé, Itajú do Colônia, Santa Cruz da Vitória, Ibicaraí, Floresta Azul, Jussari, Buerarema, São José da Vitoria, Gongogi, Barra do Rocha and Ibirataia. The total number of properties and animals by municipality was established from the IBGE database, referring to the year 2015 (IBGE, 2015) to guide the collection effort. However, the sampling was done by convenience, due to the logistical issues, such as the needed of permission from the owner for collection, horse available and personality, ability of installation and displacement of the

researchers, geographical extension range of the study (as 12 municipalities) and access facility of the farm. In that way, the maximum number of properties sampled reached 56, being at least three per municipality. In addition, the number of animals per properties or municipality was conditioned also according to the logistical issues already mentioned.

All the properties were georeferenced with a GPS device to assess the distribution of the premises and the distribution of the health status of the animals.

The horses were selected regardless of gender, breed, age and economic purpose. The number of equines sampled per property was defined for convenience (availability and behavior of the animals).

Blood samples (from 5 to 10 ml) were collected from the equines through puncture of the jugular vein and packed in siliconized tubes containing anticoagulant (EDTA), and maintaining at 5 °C for up to 5 hours until centrifugation, for 10 minutes, at 900 G. The serum was separated and stored in microtubes at -20 °C until serology was performed.

This project was developed after approval by the Ethics Committee for the Use of Animals (CEUA) of the State University of Santa Cruz - UESC, with Process no. 002/2013 and no. 09/2018, having respected and followed all the recommended ethical principles regarding the use of animals in experiments.

Gathering information using the interview form

To determine the factors associated with *S. neurona* seropositivity, a structured interview was conducted with the owners or those responsible for the farms. Objective questions included topics related to the main activity of the property; purpose of the equines; age; sex; interaction with other animals; sanitary, nutritional and reproductive management; observation of clinical neurological signs characteristic of EPM; and presence of forest area and opossums.

Detection of anti-*S. neurona* antibodies

The serological test for Indirect Fluorescent Antibody Test (IFAT) was performed with merozoites of the *S. neurona (SN138* strain), produced in cell culture. Positive and negative controls for *S. neurona* were performed on each slide. These materials were provided by Dr. Luis Fernando Pita Gondim from Federal University of Bahia -UFBA. The conjugate used was equine anti-IgG (Sigma, F7759) at a dilution of 1:600.

The sera were considered positive with the 1:80 cut-off titer when they showed complete fluorescence of *S. neurona* merozoites (Pusterla et al., 2014). Positive samples were subjected to sequential dilutions (1:80, 1:160, 1:320...), until negative results.

Didelphis spp.: capture, collection of biological material and diagnosis of *Sarcocystis* spp.

The capture of *Didelphis* spp. was performed using live traps (*Tomahawk* - 50 × 17 × 17 cm), distributed in two fragments of each sampled property. Fragment A was located in a built area, where three traps were allocated. Fragment B was located in the pasture area, where two traps were placed, therefore totaling 5 traps per property.

The traps were baited at 6:00 pm with a mixture of cornmeal, banana, oats, peanut candy, cod liver oil and sardine, and monitored daily 6:00 am for 5 consecutive days. *Didelphis* spp. found accidentally dead on the studied properties were also collected. We sampled 25 *Didelphis* spp. in which 22 animals were captured through the trap and 3 animals were found dead on the properties.

The traps with captured *Didelphis* spp. were packed in burlap bags to be transported to the physical facilities of the study areas for further handling and sample collection. *Didelphis* spp. were euthanized with intraperitoneal administration of thiopental sodium anesthetic (120 mg/kg).The anesthetic dose was calculated according to the animal weight. The anesthetic dose was calculated according to the animal weight. For each captured species, stool and intestines (small and large intestines) were collected from each animal, and stored in plastic bags, which were identified and packed and kept under refrigeration for later analysis. The animals were recorded with photographs, identified as to species and sex (Bonvicino et al., 2008). When puppies, pregnant females and lactating females were captured, it was evaluated: the presence of milk in the teats and the presence of puppies in the marsupial pouch. In view of the finding, these animals were released at the place of origin of the capture. The same release occurred with young animals.

The procedures for collecting the specimens were carried out following the ethical principles of the Brazilian College of Animal Experimentation and the Federal Council of Veterinary Medicine (CFMV). Authorization was obtained from the Biodiversity Authorization and Information System (SISBIO) under number 17131- 4 and from the Ethics Council on the Use of Animals of the State University of Santa Cruz - CEUA - UESC (Process No. 09/2018).

The stool samples were processed using a centrifuge-fluctuation technique modified from Menezes (1994). After processing the content was stored in 2.5% potassium dichromate solution for conservation.

The entire intestinal mucosa (small and large intestine) of each euthanized animal was scraped. Digestion was scraped and homogenized in distilled water with a mixer. Subsequently, 5% sodium hypochlorite was added to the mixture, which was placed in an agitator for 30 minutes at room temperature. The material was filtered in gauze and passed through serial centrifugations at 1957g for 10 minutes to remove the hypochlorite. The sediment was re-suspended in a "Hank's Balanced Salt Solution" for microscope observation.

Stool and intestinal scrapings were analyzed under an Olympus BX51 optical microscope to verify the presence of oocysts/sporocysts of *Sarcocystis* spp.

Statistical analysis

To assess the factors associated with the occurrence of the disease in horses, we identified some variables both intrinsic to the animal and the environment in which it lives, respecting the dynamics of infection by the parasite. In this sense the associations between these characteristics with the diagnosis result were evaluated using the chi-square test ($\chi 2$) and Fisher's exact test. Variables with a significance level of $p \leq 0.2$ were considered candidates for the multivariable model, including all biologically plausible bidirectional interactions. The step-by-step revision approach was used and the best fit model was defined as one that included significantly associated variables (p value <0.05) and minimum Akaike Information Criterion (AIC) value.

Variables not included in the best model were subjected to univariate analysis. All analyzes were performed on R software (R Core Team, 2017), version 3.4.2.

Results

Of the 669 horses sampled 259 were male and 410 were female; 145 were young (equines ≤ 3 years), 283 were adult (equines >3 <10 years) and 242 were senior (equines ≥ 11 years). The IFAT revealed that 53 animals (7.92%) presented IgG antibodies for *S. neurona*, with titers of 80 (n= 50), 160 (n=2) and 320 (n=1). Most farms had more than 50% of their animals sampled. Ten out of 56 farms had at least one seropositive equine (Table 1).

Table 1. Number of animals collected by municipality and by property and detection and distribution of anti-*S. neurona* antibodies in horses in municipalities in the Ilhéus-Itabuna micro-region, Bahia, Brazil.

Municipality	Number of animals (IBGE*)	Number of sampled farms	Total of horses in all farms	Total of horses sampled	Number of Posive horses	Prevalence (%) (CI 95%)
Barra do Rocha	102	5	20	14	1	7.1 (1.1-47.2)
Buerarema	410	5	74	41	6	14.6 (6.9-30.6)
Floresta Azul	800	4	261	100	6	6.0 (2.7-13.0)
Ibicarai	560	7	123	77	12	15.6 (9.2-26.2)
Gongogi	467	4	56	25	4	16.0 (6.5-39.3)
Ibirataia	104	5	44	32	0	0 (0.0 -0.0)
Itabuna	958	4	30	28	0	0 (0.0-0.0)
Itaju do Colônia	3431	4	860	186	8	4.3 (2.2-8.5)
Itapé	2881	8	334	74	6	8.1 (3.7-17.4)
Jussari	541	3	36	26	4	15.4 (6.2-37.9)
Santa Cruz da Vitória	808	3	225	54	5	9.3 (4.0-21.3)
São José	133	4	13	12	1	8.3 (1.2-54.4)
Total	**11195**	**56**	**2076**	**669**	**53**	7.9 (6.1-10.2)

*According to Brazilian Institute of Geography and Statistic (IBGE, 2015); CI: Confidence interval.

Of the seropositive equines, 9.70% (25) were male and 6.80% (28) female; 7.58% (11) were young, 6.77% (19) adult and 9.50% (23) senior. There was no significant difference between sex or age range (Table. 2). The purposes of trade and work were significant ($p<0.05$), such that being used for the purpose of work (21.6%), was a risk factor (OR>1) and being used for the purpose of trade (3.6%) was a protective factor. There was no association between seropositivity of horses as to their purpose for sport and commerce, type of feeding, management of the stall, management of pasture and presence of neurological signs (Table 2).

Table 2. Stratified seroprevalence of *S. neurona* in equines sampled from the Ilhéus-Itabuna microregion, Bahia, Brazil, and results of weighted logistic regression analysis of possible risk factors for seropositivity.

Variables	Description	Seropositive		Seronegative		Multivariate Analysis		Univariate Analysis	
		N	(%)	N	(%)	OR Adj (95%CI)	p value	OR Adj (95%CI)	p value
Purpose	Trade	2	3.6	53	96.4		<0.001		
	Reproduction	18	6.8	248	93.2	1.66 (0.37-7.5)	0.511		
	Sport	8	5.8	129	94.2	1.19 (0.24-6)	0.833		
	Work	25	21.6	91	78.4	5.08 (1.11-23.33)	0.037		
	WI	0	0.0	95	100.0				
Type of food	Roughage only	10	7.3	127	92.7			0.75 (0.29-1.92)	0.548
	Concentrate only	0	0.0	2	100.0			0 (0,Inf)	0.984
	roughage and concentrate	33	7.2	427	92.8				0.093
	WI	10	14.3	60	85.7				
Age	Young	11	7.6	133	92.4			1.22 (0.56-2.65)	0.614
	Adult	19	6.7	264	93.3				0.381
	Senior	23	9.5	219	90.5			1.57 (0.83-2.97)	0.167
Open horse stall	Yes	8	9.5	76	90.5				
	No	45	7.7	540	92.3			1.06 (0.48-2.33)	0.891
Forage	Yes	43	7.2	552	92.8			0.6 (0.29-1.26)	0.197
	No	10	13.5	64	86.5				
Hay	Yes	7	3.8	179	96.2	0.47 (0.2-1.13)	0.074		
	No	46	9.5	437	90.5				
Gender	Male	28	6.8	382	93.2			1.68 (0.95-2.97)	0.17
	Female	25	9.7	234	90.3				
Horses exhibition event	Yes	4	7.4	50	92.6			0.77 (0.27-2.22)	0.616
	No	49	8.0	566	92.0				
Local	Horse stall only	5	5.5	86	94.5			334756.56 (0,Inf)	0.988
	Pasture only	40	8.1	451	91.9			645133.09 (0,Inf)	0.987
	Horse stall and pasture	8	9.5	76	90.5			614166.7 (0,Inf)	0.988
	WI	0	0.0	3	100.0				
Number of animals on the farm	1-5	38	9.0	383	91.0			0.75 (0.52-1.09)	0.106
	15-30	10	7.0	132	93.0				
	20-45	3	5.2	55	94.8				
	>45	2	4.2	46	95.8				
Neurological signs	Yes	2	11.0	50	96.2			1.82 (0.39-8.43)	0.474
	No	51	510.0	566	91.7				

WI = Without Information; OR Adj = Odds Ratio Adjusted; Inf = Infinity; N = Number of seropositive animals; p value = probability of observing a difference greater than that observed under the null hypothesis, considering significant when less than 5%.

Among the analyzed municipalities, Gongogi presented the highest occurrence of seropositive horses (16.0%), whereas there were no positive animals for anti- *S. neurona* antibodies in the municipalities of Ibirataia and Itabuna (Figure 1; Table 1). There was no statistical difference among them.

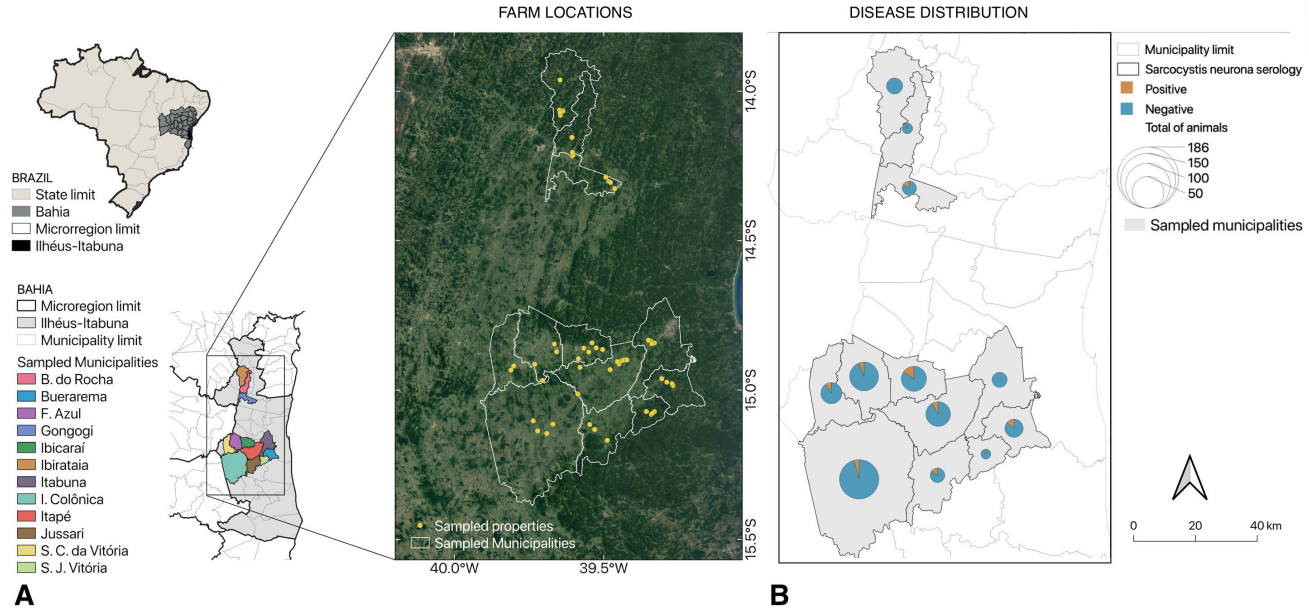

Figure 1. Sampled farms and proportion of seropositive horses for *S. neurona* in the sampled municipalities of the Microregion of Ilhéus and Itabuna in the state of Bahia - Brazil. A) Sampled properties in each municipalities of Ilhéus-Itabuna Microrregion in Bahia State of Brazil; B) Proportion of seropositive and seronegative animals in each sampled points.

A total of 25 *Didelphis* spp. was captured, being 10 females (40%) and 15 males (60%), 60% (15/25) belonging to the *D. aurita* species and 40% (10/25) to *D. albiventris.*

Didelphis spp. was captured in 75% of the municipalities, and distributed as follows: Jussari (1/25); Buerarema (1/25); Gongogi (1/25); Ibicaraí (3/25); Itabuna (3/25); Santa Cruz da Vitoria (3/25); Ibirataia (3/25); Itapé (4/25); and Floresta Azul (6/25). It was observed that 52 from the 56 properties in the study, were bordered or possessed forest or bush area. At least one *Didelphis* spp. was captured on 18 from the 56 properties.

Of the 18 properties where *Didelphis* spp. were captured, 6 (33,3%) presented equines seropositive in the IFAT. Of the 38 properties that had no successful captures, 25 (65.70%) presented positive equines. However, the association between the presence of *Didelphis* spp. on the properties and positive equines was not statistically significant, also there was no statistical significance for those properties that border or possess forest or bush area with *S. neurona* positive equines.

Of the 25 *Didelphis* spp. captured, all were negative for the presence of oocysts and sporocysts in the analyzed stool samples and scraping of small and large intestines. It is worth noting that the low number of animals captured was due to restriction imposed by the Ethics Committee for the Use of Animals (CEUA) of the Santa Cruz State University-UESC.

Discussion

The seroprevalence for *S. neurona* antibodies observed in the equines sampled in this study (7.92%) was similar to that described in Alagoas state, northeast Brazil (2.8%) (Valença et al., 2019), but considerably low compared to the numbers observed in Minas Gerais state, southeast Brazil (26.0%) (Ribeiro et al., 2016), and in the United States (27,6%) (Pusterla et al., 2014). All the above studies used the same serological test and cut-off values as the present study. The IFAT is an excellent choice, with a sensitivity of 94% and specificity of 88.9%. The test allows quantifying the infection through the final title of it (Duarte et al., 2003; Johnson et al., 2013).

The prevalence of *S. neurona* in horses is related to several intrinsic factors (physical, immunological condition and recent stress history) and extrinsic factors (environmental conditions and proximity to areas of occurrence of

the definitive positive host), but which will determine high or low prevalence is the direct exposure of the horse to the *S. neurona* through feces of positive *Didelphis* spp.

In the present study, the 25 *Didelphis* spp. captured in the properties were negative for *Sarcocystis* spp. No sporocysts were found in the intestinal scrapings of all 39 examined opossums in Bahia, however *Sarcocystis* spp. DNA was identified in tissues of 16/39 (41%) animals (Gondim et al., 2017). *S. neurona*, that had been reported in Brazil infecting South American opossum *D. albiventris* (Dubey et al., 2001b), but has never been genetically confirmed in the country. *S. lindsayi* have been reported in *D. aurita*, but most isolates of *Sarcocystis* spp. derived from opossums reported in Brazil are *S. falcatula*-like organisms, as they are infective to birds (Stabenow et al., 2012; Valadas et al., 2016; Cesar et al., 2018; Gallo et al., 2018).

The relationship of *Didelphis* spp. and *Sarcocystis* spp. may be associated with some behavioral characteristics of skunks, as it is an omnivorous animal with general eating habits, since their diet is composed of a wide range of items including invertebrates, small vertebrates, eggs, brans, birds, fruits, carcasses and occasionally carrion, flowers, nectar and tree gum (Aléssio et al., 2005; Santori & Moraes, 2006). Thus, changes in diet lead to the possibility of negative animals since contamination occurs through the ingestion of *Sarcocystis* present in the muscle tissues of intermediate hosts.

The Atlantic Forest region has a great diversity of food for opossums, with a large quantity and variety of fruits and insects. Such availability diminishes the need to search for other types of food, such as intermediary hosts that are part of the biological cycle, since this species is omnivorous. This fact contributes to a lower probability of *Didelphis* spp. being infected with *S. neurona*, and, therefore, corroborating the low prevalence of infected species in the present study.

Another fact that corroborates the negativity of *Didelphis* spp. is its presence at some time on the studied properties, being observed on 92.86% (52/56) of the properties. This fact may be associated with the synanthropic characteristics of *Didelphis* spp. Opossums adapt easily to *human* coexistence, colonizing houses and their surroundings, taking advantage of shelter because they adapt to house linings and hollow trees, have access to food (household food waste, bird breeding, animal feed) and water availability (Andrade et al., 2002). This evidence of the migration of these animals onto the properties may correlate with the ease and unrestricted supply of food other than intermediate hosts. Although no opossums were found positive in this study, as a reduced number of animals was used, other opossums may be positive for *S.neurona* and contaminate the environment with feces containing sporocysts.

According to Dubey et al. (2015), an animal breeding servant can influence the possibility of the animal becoming infected. This corroborates the data found in this study. The use for work purposes (21.6%) was a risk factor. It is worth considering that within this topic "work" included in this variable, it considers horses that make trips transporting cattle to other properties, perform routine work inside the property, are used to go in the city as a means of transport. This makes it possible for horses to be exposed to different environments on the property, as well as other rural environments, at different times of the year, increasing the scope for interaction with the environment, other farms, having access to various foods such as chopped grass, hay, pastures, as well as access as pasture areas to the forest margin, and these environments may be contaminated with *S. neurona* sporocysts. Another important fact is that the equines remain on the property for many years, with a life expectancy of 20 years. This enables long-term interaction with *Didelphis* spp., which predisposes the equines to *S. neurona* infection (Morley et al., 2008).

Being used for the purpose of trade (3.6%) was considered a protective factor. This fact may be associated with the short time these animals spend on the property, since commercialization is rotational, as these animals present characteristics desired by the market such as breed, coat color, age, height, temperament and potential usability. Another relevant aspect is the management of the animals destined for trade; on most of the properties, the hostlers provided special care regarding the cleaning of the stalls, individual pickets per animal and directed food management.

Although Bentz et al. (2003) and Duarte et al. (2004) reported a direct association between age and seropositivity for *S. neurona*, the present study did not prove this relationship, corroborating the results of Pivoto et al. (2014) and Ribeiro et al. (2016). There was no association between seropositivity of horses and sex, which is in agreement with the findings of Blythe et al. (1997).

Conclusion

Horses raised in the south of Bahia mesoregion, in the Ilhéus-Itabuna microregion, have a low anti-*S. neurona*. The fact that these animals are destined for work is considered a risk factor as well as equine care for trade is a protective factor. *Didelphis* spp. sampled in the present study were negative for *Sarcocystis* spp.

Acknowledgements

This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB) for scholarship and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (grant 306308/2015-0). The authors thank Dr. Luis Fernando Pita Gondim for merozoites of the *S.neurona 138 (SN138)* strain.

References

Aléssio FM, Mendes Pontes AR, Silva VL. Feeding by *Didelphis albiventris* on tree gum in the northeastern Atlantic Forest of Brazil [online]. *Mastozool Neotrop* 2005; 12(1): 53-56 [cited 2020 Nov 2020]. Available from: https://www.redalyc.org/articulo.oa?id=45712105

Andrade A, Pinto SC, Oliveira RS. *Animais de laboratório: criação e experimentação*. Rio de Janeiro: Editora FIOCRUZ; 2002. 388 p. https://doi.org/10.7476/9788575413869.

Barros CSL, Barros SS, Santos MN, Silva CAM, Waihrich F. Mieloencefalite eqüina por protozoário. *Pesq Vet Bras* 1986; 6(2): 45-49.

Bentz BG, Ealey KA, Morrow J, Claypool PL, Saliki JT. Seroprevalence of antibodies to *Sarcocystis neurona* in equids residing in Oklahoma. *J Vet Diagn Invest* 2003; 15(6): 597-600. http://dx.doi.org/10.1177/104063870301500617. PMid:14667028.

Blythe LL, Granstrom DE, Hansen DE, Walker LL, Bartlett J, Stamper S. Seroprevalence of antibodies to *Sarcocystis neurona* in horses residing in Oregon. *J Am Vet Med Assoc* 1997; 210(4): 525-527. PMid:9040840.

Bonvicino CR, Oliveira JA, D'Andrea PS. *Guia dos roedores do Brasil, com chaves para gêneros baseadas em caracteres externos*. Rio de Janeiro: Centro Pan-Americano de Febre Aftosa-OPAS/OMS; 2008.

Cesar MO, Matushima ER, Zwarg T, Oliveira AS, Sanches TC, Joppert AM, et al. Multilocus characterization of *Sarcocystis falcatula*-related organisms isolated in Brazil supports genetic admixture of high diverse SAG alleles among the isolates. *Exp Parasitol* 2018; 188: 42-49. http://dx.doi.org/10.1016/j.exppara.2018.03.004. PMid:29522766.

Duarte PC, Daft BM, Conrad PA, Packham AE, Gardner IA. Comparison of a serum indirect fluorescent antibody test with two western blot tests for the diagnosis of the equine protozoal myeloencephalitis. *J Vet Diagn Invest* 2003; 15(1): 8-13. http://dx.doi.org/10.1177/104063870301500103. PMid:12580288.

Duarte PC, Daft BM, Conrad PA, Packham AE, Saville WJ, MacKay RJ, et al. Evaluation and comparison of an indirect fluorescent antibody test for detection of antibodies to *Sarcocystis neurona*, using serum and cerebrospinal fluid of naturally and experimentally infected, and vaccinated horses. *J Parasitol* 2004; 90(2): 379-386. http://dx.doi.org/10.1645/GE-3263. PMid:15165063.

Dubey JP, Howe DK, Furr M, Saville WJ, Marsh AE, Reed SM, et al. An update on *Sarcocystis neurona* infections in animals and equine protozoal myeloencephalitis (EPM). *Vet Parasitol* 2015; 209(1-2): 1-42. http://dx.doi.org/10.1016/j.vetpar.2015.01.026. PMid:25737052.

Dubey JP, Lindsay DS, Kerber CE, Kasai N, Pena HFJ, Gennari SM, et al. First isolation of *Sarcocystis neurona* from the South American opossum, *Didelphis albiventris*, from Brazil. *Vet Parasitol* 2001a; 95(2-4): 295-304. http://dx.doi.org/10.1016/S0304-4017(00)00395-2. PMid:11223209.

Dubey JP, Lindsay DS, Saville WJA, Reed SM, Granstrom DE, Speer CA. A review of *Sarcocystis neurona* and equine protozoal myeloencephalitis (EPM). *Vet Parasitol* 2001b; 95(2-4): 89-131. http://dx.doi.org/10.1016/S0304-4017(00)00384-8. PMid:11223193.

Dubey JP, Lindsay DS. Isolation in immunodeficient mice of *Sarcocystis neurona* from opossum (*Didelphis virginiana*) faeces, and its differentiation from *Sarcocystis falcatula. Int J Parasitol* 1998; 28(12): 1823-1828. http://dx.doi.org/10.1016/S0020-7519(98)00166-0. PMid:9925260.

Dubey JP, Miller S. Equine protozoal myeloencephalitis in a pony. *J Am Vet Med Assoc* 1986; 188(11): 1311-1312. PMid:3721987.

Faria TTR, Pessoa GO, Bilhrer DA, Lima APL, Varaschin MS, Sousa TM. Mieloencefalite protozoária equina de evolução clínica aguda: relato de caso. Lavras. *Pubvet* 2017; 11(1): 40-45. http://dx.doi.org/10.22256/pubvet.v11n1.40-45.

Gallo SSM, Lindsay DS, Ederli NB, Matteoli FP, Venancio TM, de Oliveira FCR. Identification of opossums *Didelphis aurita* (Wied-Neuweid, 1826) as a definitive host of *Sarcocystis falcatula*-like sporocysts. *Parasitol Res* 2018; 117(1): 213-223. http://dx.doi.org/10.1007/s00436-017-5695-4. PMid:29192336.

Gondim LSQ, Jesus RF, Ribeiro-Andrade M, Silva JC, Siqueira DB, Marvulo MF, et al. *Sarcocystis neurona* and *Neospora caninum* in Brazilian opossums (*Didelphis* spp.): molecular investigation and *in vitro* isolation of *Sarcocystis* spp. *Vet Parasitol* 2017; 243: 192-198. http://dx.doi.org/10.1016/j.vetpar.2017.07.002. PMid:28807293.

Henker LC, Bandinelli MB, Andrade CP, Bianchi MV, Sonne L, Driemeier D, et al. Pathological, immunohistochemical, and molecular findings of equine protozoal myeloencephalitis due to *Sarcocystis neurona* infection in Brazilian horses. *Trop Anim Health Prod* 2020; 52(6): 3809-3817. http://dx.doi.org/10.1007/s11250-020-02419-y. PMid:33011934.

Hoane JS, Gennari SM, Dubey JP, Ribeiro MG, Borges AS, Yaile LE, et al. Prevalence of *Sarcocystis neurona* and *Neospora* spp. infection in horses from Brazil based on presence of serum antibodies to parasite surface antigen. *Vet Parasitol* 2006; 136(2): 155-159. http://dx.doi.org/10.1016/j.vetpar.2005.10.023. PMid:16310955.

Instituto Brasileiro de Geografia e Estatística – IBGE. *Disponível em Tabela 3939: efetivo dos rebanhos, por tipo de rebanho* [online]. Salvador; 2015 [cited 2017 Jan 20]. Available from: https://sidra.ibge.gov.br/geratabela?name=Tabela%201.xlsx&format=xlsx&medidas=true&query=t/3939/g/2/v/all/p/2015/c79/all/l/p%2Bv,c79,t" https://sidra.ibge.gov.br/geratabela?name=Tabela%201.xlsx&format=xlsx&medidas=true&query=t/3939/g/2/v/all/p/2015/c79/all/l/p%2Bv,c79,t

Instituto Brasileiro de Geografia e Estatística – IBGE. *Produção da Pecuária Municipal* [online] 2016 [cited 2018 Jan 15]. Available from: https://biblioteca.ibge.gov.br/visualizacao/periodicos/84/ppm_2016_v44_br.pdf

Johnson AL, Morrow JK, Sweeney RW. Indirect fluorescent antibody test and surface antigen ELISAs for antemortem diagnosis of equine protozoal myeloencephalitis. *J Vet Intern Med* 2013; 27(3): 596-599. http://dx.doi.org/10.1111/jvim.12061. PMid:23517480.

Lima RAS, Cintra AG. *Revisão do estudo do complexo do agronegócio do cavalo* [online]. Brasília: Ministério da Agricultura, Pecuária e Abastecimento; 2016 [cited 2018 Fev 05]. Available from: https://www.gov.br/agricultura/pt-br/assuntos/camaras-setoriais-tematicas/documentos/camaras-setoriais/equideocultura/anos-anteriores/revisao-do-estudo-do-complexo-do-agronegocio-do-cavalo/@@download/file/revisao-do-estudo-do-complexo-do-agronegocio-do.pdf

Lins LA, Frey F Jr, Berne MEA, Nogueira CEW. Mieloencefalite protozoária eqüina em equinos nativos do município de Bagé-RS, sul do Brasil. *RPCV* 2008; 103(567-568): 177-180.

MacKay R. Mieloencefalopatia protozoária eqüina. In: Aiello SE, editor. *Manual Merck de veterinária*. 8. ed. São Paulo: Roca; 2001. p. 771-772.

Masri MD, Lopez de Alda J, Dubey JP. *Sarcocystis neurona*-associated ataxia in horses in Brazil. *Vet Parasitol* 1992; 44(3-4): 311-314. http://dx.doi.org/10.1016/0304-4017(92)90128-V. PMid:1466140.

Menezes RCAA. *Epidemiologia da Eimeria Arloing (Marotel, 1905) Martan, 1909 (Apicomplexa: Eimeriidae) na microrregião serrana fluminense, estado do Rio de Janeiro* [dissertação]. Seropédica: Universidade Federal Rural do Rio de Janeiro; 1994.

Morley PS, Traub-Dargatz JL, Benedict KM, Saville WJA, Voelker LD, Wagner BA. Risk factors for owner-reported occurrence of equine protozoal myeloencephalitis in the US equine population. *J Vet Intern Med* 2008; 22(3): 616-629. http://dx.doi.org/10.1111/j.1939-1676.2008.0082.x. PMid:18466255.

Pivoto FL, Macêdo AG Jr, Silva MV, Ferreira FB, Silva DAO, Pompermayer E, et al. Serological status of mares in parturition and the levels of anti-bodies (IgG) against protozoan family Sarcocystidae from their pre colostral foals. *Vet Parasitol* 2014; 199(1-2): 107-111. http://dx.doi.org/10.1016/j.vetpar.2013.10.001. PMid:24183649.

Pusterla N, Tamez-Trevino E, White A, VanGeem J, Packham A, Conrad PA, et al. Comparison of prevalence factors in horses with and without seropositivity to *Neospora hughesi* and/or *Sarcocystis neurona. Vet J* 2014; 200(2): 332-334. http://dx.doi.org/10.1016/j.tvjl.2014.03.014. PMid:24703324.

R Core Team. *R: a language and environment for statistical computing* [online]. Vienna: R Foundation for Statistical Computing; 2017 [cited 2018 Mar 15] Available from: https://www.R-project.org/

Reed M, Bayly M. *Medicina interna eqüina*. 1. ed. Rio de Janeiro: Guanabara Koogan; 2000.

Ribeiro MJM, Rosa MHF, Bruhn FRP, Garcia AM, Rocha CMBM, Guimarães AM. Seroepidemiology of *Sarcocystis neurona, Toxoplasma gondii* and *Neospora* spp. among horses in the south of the state of Minas Gerais, Brazil. *Rev Bras Parasitol Vet* 2016; 25(2): 142-150. http://dx.doi.org/10.1590/S1984-29612016029. PMid:27334814.

Santori RT, Moraes DA. Alimentação, nutrição e adaptações alimentares de marsupiais brasileiros. In: Monteiro-Filho ELA, Cáceres NT, editors. *Os marsupiais do Brasil: biologia, ecologia e evolução*. Campo Grande: Editora UFMS; 2006. p. 241-254.

Silva DP, Borges AS, Amorim RM, Graf-Kuchembuck MR, Gonçalves RC, Chiacchio SB. Mieloencefalite protozoária equina: revisão de literatura. *Rev Cons Fed Med Vet* 2003; 9(28-29): 34-40.

Smith BP. *Tratado de medicina interna de grandes animais*. 2. ed. São Paulo: Manole; 1994.

Stabenow CS, Ederli NB, Lopes CW, Oliveira FC. *Didelphis aurita* (Marsupialia: Didelphidae): a new host for *Sarcocystis lindsayi* (Apicomplexa: Sarcocystidae). *J Parasitol* 2012; 98(6): 1262-1265. http://dx.doi.org/10.1645/GE-3140.1. PMid:22571294.

Thomassian A. *Enfermidades dos cavalos*. 4. ed. São Paulo: Livraria Varela; 2005.

Valadas SY, da Silva JI, Lopes EG, Keid LB, Zwarg T, de Oliveira AS, et al. Diversity of *Sarcocystis* spp. shed opossums in Brazil inferred with phylogenetic analysis of DNA coding ITS1, cytochrome B, and surface antigens. *Exp Parasitol* 2016; 164: 71-78. http://dx.doi.org/10.1016/j.exppara.2016.02.008. PMid:26905780.

Valença SRFA, Ribeiro-Andrade M, Moré G, Albuquerque PPF, Pinheiro JW Jr, Mota RA. Low prevalence of infection by *Sarcocystis neurona* in horses from the State of Alagoas, Brazil. *Rev Bras Parasitol Vet* 2019; 28(2): 298-302. http://dx.doi.org/10.1590/s1984-29612019027. PMid:31188947.

Vardeleon D, Marsh AE, Thorne JG, Loch W, Young R, Johnson PJ. Prevalence of *Neospora hughesi* and *Sarcocystis neurona* antibodies in horses from various geographical locations. *Vet Parasitol* 2001; 95(2-4): 273-282. http://dx.doi.org/10.1016/S0304-4017(00)00393-9. PMid:11223207.