

Cryptosporidium spp. infection in mares and foals of the northwest region of São Paulo State, Brazil

Infecção por *Cryptosporidium* spp. em éguas e potros da região noroeste do Estado de São Paulo, Brasil

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Abstract

The present study aimed to analyze the occurrence of infection by *Cryptosporidium* spp. in mares and their respective foals. This study was carried out in 11 farms located in the municipalities of Araçatuba, Birigui, Guararapes and Santo Antônio do Aracangua, in the northwest region of the State of São Paulo, from November 2010 to March 2011. A total of 98 mares and 98 foals of several breeds were analyzed; among foals, 59 were males and 39 females, aged from three to 330 days. Feces were collected directly from the rectal ampulla, purified and processed according to modified Kinyoun stain. Occurrence of *Cryptosporidium* spp. was 21.4% (21/98) for foals and 18.4% (18/98) for mares. Occurrence of *Cryptosporidium* spp. had significant association with breeds and age of animals. Results obtained led to the conclusion that foals older than two months and Mangalarga animals are less susceptible to the occurrence of *Cryptosporidium* spp.

Keywords: *Cryptosporidium*, frequency, mares, foals.

Resumo

O presente estudo teve como objetivo analisar a ocorrência da infecção por *Cryptosporidium* spp. em éguas e seus respectivos potros. Este estudo foi realizado em 11 fazendas localizadas nos municípios de Araçatuba, Birigui, Guararapes e Santo Antônio do Aracangua, na região Noroeste do Estado de São Paulo, de novembro de 2010 a março de 2011. Um total de 98 éguas e 98 potros de diversas raças foram analisados, sendo que, entre os filhotes, 59 eram machos e 39 fêmeas, cujas idades variavam de três até 330 dias. Fezes foram colhidas diretamente da ampola retal, purificadas e processadas pela técnica de Kinyoun modificada. A ocorrência de *Cryptosporidium* spp. observada foi de 21,4% (21/98) para potros e 18,4% (18/98) para éguas. A ocorrência de *Cryptosporidium* spp. teve uma associação significativa com a raça e a idade dos animais. A partir dos resultados obtidos, conclui-se neste estudo que potros com idade superior a dois meses e animais da raça Mangalarga foram menos susceptíveis à ocorrência de *Cryptosporidium* spp.

Palavra-chave: *Cryptosporidium*, frequência, éguas, potros.

Introduction

Birds, amphibians, reptiles and mammals, including humans, can be different host species for *Cryptosporidium* spp. (FAYER, 2010; SMITH; NICHOLS, 2010; PLUTZER; KARANIS, 2009).

The protozoa *Cryptosporidium* spp. is included in the Neglected Diseases Initiative of the World Health Organization (WHO) due to their close relationship with deficient sanitation and low income of the population; thus, they are considered responsible for child malnutrition and death (THOMPSON et al., 2008).

Fecal oocysts of *Cryptosporidium* spp. released by equine may represent a source of human infection and are resistant to the environmental conditions (PLUTZER; KARANIS, 2009).

Evaluation of the occurrence of *Cryptosporidium* spp. in horses has drawn the attention of research worldwide (BURTON et al., 2010; COLE et al., 1998; JOHNSON et al., 1997; MAJEWSKA et al., 2004; MARQUES, 2010; SOUZA et al., 2009; VERONESI et al., 2009). Positive fecal samples for *Cryptosporidium parvum* oocysts were significantly greater for foals under six months of age and thoroughbreds compared to other breeds, in Texas (COLE et al., 1998).

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Few studies have been carried out in Brazil (MARQUES, 2010; SOUZA et al., 2009). Therefore, data on the occurrence of *Cryptosporidium* spp. in equine species are still scarce.

The protozoan *Cryptosporidium* spp. has been associated with the presence of diarrhea in immunocompetent and immunodeficient animals (BJORNEBY et al., 1991; COLEMAN et al., 1989; GIBSON et al., 1983; GRINBERG et al., 2003; MAIR et al., 1990). Nevertheless, some clinical and epidemiological aspects of this infection are still not clear (VERONESI et al., 2009).

Meireles (2010) discussed this situation in researches carried out in Brazil on domestic and wild animals infected with *Cryptosporidium* spp. demonstrating the increasing amount of works on this theme.

The present research aimed to analyze the occurrence of infection by *Cryptosporidium* spp. in mares and their respective foals in municipalities of the northwest region of the State of Sao Paulo and to correlate the positivity for this parasite with the variables breed, sex, age, degree of fecal hydration, and water source.

Materials and Methods

This study was approved by the Ethics Committee for Animal Use of the Dentistry School of UNESP, Araçatuba Campus; protocol number 2009-002165.

This study was carried out in 11 farms located in the municipalities of Araçatuba, Birigui, Guararapes and Santo Antônio do Aracangua, in the northwest region of the State of Sao Paulo, from November 2010 to March 2011. A total of 98 mares and their respective foals were investigated. Breeds were as follows: 50.51% (50/98) Quarter Horse, 42.42% (42/98) Mangalarga Marchador, 5.05% (5/98) Paint Horse, and 1.01% (1/98) Crioula; among foals, 59 were males and 39 females, aged between three and 330 days. Foals were allocated, according to age, in two groups: ≤ 60 days and > 60 days.

An epidemiological questionnaire was applied to the owners, addressing questions related to age, sex, breed and types of water source for the equines involved in the research.

Fecal samples were collected directly from the rectal ampulla of animals and stored under refrigeration; subsequently, fecal smears were prepared. To standardize the determination of the degree of fecal hydration, the following classification was adopted: solid, semi-solid, pasty and liquid. The following established criterion was used regarding the color: green, mossy green, brown, yellowish green and yellow. Information about fecal conditions was introduced in the data bank to correlate the physical status of feces to the occurrence of the parasite, also including the variables: age, sex, breed, and types of water source (river, pond, millpond, draw-well, water tank) used by the animals involved in this study.

Fecal smears were prepared by centrifugation-sedimentation technique in water and ether (MELONI; THOMPSON, 1996) used in the routine laboratory and, subsequently, stained according to the modified Kinyoun technique (LENNETTE et al., 1985) for observation of *Cryptosporidium* spp. oocysts.

A light optical microscope, 40 \times and 100 \times magnification, was employed for observation. Infection by *Cryptosporidium* spp. was classified into positive or negative, based on the presence or absence of oocysts in one sample from each animal.

Variables were analyzed according to the Chi-square test (χ^2) or Fisher's exact test and Mann-Whitney test (ZAR, 1999), employing SAS software (SAS, 2008), at 5% significance level.

Results and Discussion

Occurrence of *Cryptosporidium* spp. was detected, using Kinyoun stain, in 21.4% (21/98) of the foals and 18.4% (18/98) of the mares analyzed. Foals presented higher occurrence of *Cryptosporidium* spp. compared to adult mares, which corroborates the findings by Burton et al. (2010).

There was positivity of 12.2% (12/98) of samples for both foals and their respective mothers; however, there was no significant association. Mature horses are not considered important sources of infection for foals (COLE et al., 1998) and the infection of foals was not associated with that of their mothers (MAJEWSKA et al., 2004).

Compared to the occurrence of infection by *Cryptosporidium* spp. in the equines detected herein, studies have described lower frequencies: 0% (0/91) by Johnson et al. (1997) in California, USA and 0.75% (3/396) by Souza et al. (2009) in Rio de Janeiro, Brazil. However, a higher frequency of 27.8% (25/90) of *Cryptosporidium* spp. infection was obtained using Ziehl-Neelsen staining technique by Marques (2010) in Porto Alegre, Brazil. Thus, asymptomatic adult horses can be considered sources of environmental contamination due to the fecal content they produce (MARQUES, 2010).

Foals younger than two months were more susceptible to *Cryptosporidium* spp. infection ($p = 0.0069$), with 37.5% (12/32) occurrence, while the group older than 60 days showed 13.6% (9/66) of positive samples (Table 1). Two positive samples from 60 and 90-day-old foals were liquid feces and one positive mother presented feces of pasty consistency.

Cole et al. (1998) reported diarrhea related to the presence of the above-mentioned protozoan, and Grinberg et al. (2003) seemed to have evidenced this clinical sign in immunocompetent foals. In the present study, the consistency ($p = 0.2119$ and $p = 0.2403$) and color ($p = 0.6842$ and $p = 0.5023$) of feces were not associated with the occurrence of this disease in foals and mares, respectively, which agrees with the reports by Veronesi et al. (2009) and Burton et al. (2010).

As regards the breed, there was a significant association ($p < 0.0001$) between the occurrence of *Cryptosporidium* spp. and the breed of foals, with 30.0% (15/50) positivity for Quarter Horse, 2.4% (1/42) for Mangalarga Marchador, 80.0% (4/5) for Paint Horse, and 100% (1/1) for Crioula (Table 1). However, Cole et al. (1998) reported non-significant association for Thoroughbred horses.

In this study, there was no significant association between the protozoan occurrence and the sex of foals ($p = 0.1838$) (Table 1); the same was found for the sources of water drunk by the animals in the farm ($p = 0.4877$).

Souza et al. (2009) noted higher proportion of males naturally infected by *Cryptosporidium* spp. and concluded that the disease in equines, with different management types, is probably related to the immunological state and sanitary conditions found in the place where they live, since they were already considered sources of environmental infection (VERONESI et al., 2009). Concerning

Table 1. Occurrence of infection by *Cryptosporidium* spp. by modified Kinyoun stain according to the variables breed, sex and age from 98 foals in the northwest region of the State of Sao Paulo, 2012.

Variable	Category	n	Occurrence		P-value
			Positive	%	
Breed	Quarter Horse	50	15	30,0	<0.0001 ⁽¹⁾
	Mangalarga Marchador	42	1	2,4	
	Paint Horse	5	4	80,0	
	Crioula	1	1	100,0	
Sex	Male	59	10	16,9	0.1838 ⁽²⁾
	Female	39	11	28,2	
	Total	98	21	21,2	
Age (days)	≤60	32	12	37,5	0.0069 ⁽²⁾
	>60	66	9	13,6	

⁽¹⁾Fisher's exact test; ⁽²⁾Chi-square.

this aspect, it must be highlighted that, in previous epidemiological studies, *Cryptosporidium* spp. infection was concentrated on one farm or site, and this may be due to the characteristics of the management adopted in the farm (VERONESI et al., 2009; BURTON et al., 2010).

Conclusion

The results obtained led to the conclusion that foals older than two months and the breed Mangalarga were less susceptible to the occurrence of *Cryptosporidium* spp.

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