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Prevalence of infection by *Cryptosporidium* spp. in calves and associated risk factors in Northeastern Brazil

[*Prevalência da infecção por* Cryptosporidium *spp. e fatores de risco associados em bezerros do Nordeste do Brasil*]

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ABSTRACT

Cryptosporidium spp. are zoonotic protozoa, frequently associated with diarrhea in calves, which are responsible for important economic losses. The aim of this study was to assess the prevalence of infection by *Cryptosporidium* spp. and its associated risk factors among calves raised in a milk production region of Northeastern Brazil. Fecal samples (n = 385) were obtained from young animals (up to ten months old) and evaluated by means of centrifugal fecal sedimentation in formalin-ether followed by the modified Ziehl-Neelsen staining technique. In addition, *Odds Ratio* (OR) was calculated to evaluate associations between variables and infection by these protozoa. Out of all samples analyzed, 25.7% (99/385) scored positive for the presence of *Cryptosporidium* spp. Contact with other species (goat and sheep) (OR = 3.33; p = 0.000), use of a semi-intensive rearing system (OR = 1.70; p = 0.024) and absence of hygienic conditions (fecal contamination of food and water) (OR = 1.64; p = 0.029) were considered to be risk factors. Data herein reported shows that the implementation of hygienic-sanitary measures on the farms studied, it is imperative to reduce *Cryptosporidium* spp. infection and consequently the economic impact caused by this pathogen.

Keywords: calves, *Cryptosporidium* spp., protozoan, risk factors

RESUMO

Cryptosporidium *spp. são protozoários zoonóticos frequentemente associados à diarreia em bezerros e responsáveis por importantes perdas econômicas. O objetivo deste estudo foi avaliar a prevalência e os fatores de risco associados à infecção por* Cryptosporidium *spp. em bezerros de propriedades leiteiras no Nordeste do Brasil. Amostras fecais (n = 385) foram obtidas de animais jovens (até 10 meses de idade) e avaliadas por centrífugo-sedimentação em formol éter, seguida da técnica de coloração de Ziehl-Neelsen modificada. A* Odds Ratio (OR) *foi calculada para avaliar a associação entre variáveis e infecção pelos protozoários. De todas as amostras analisadas, 25,7% (99/385) apresentaram oocistos de* Cryptosporidium *spp. Contato com outras espécies (caprino e ovino) (OR = 3,33; p = 0,000), sistema semi-intensivo de criação (OR = 1,70; p = 0,024) e ausência de condições higiênicas (contaminação fecal do alimento e da água) (OR = 1,64; p = 0,029) foram considerados fatores de risco. Com base nos resultados, é imprescindível a adoção de medidas higiênico-sanitárias nas fazendas estudadas, a fim de reduzir infecção por* Cryptosporidium *spp. e o impacto econômico causado por esse patógeno.*

Palavras-chave: bezerros, Cryptosporidium *spp., protozoários, fatores de risco*

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INTRODUCTION

Cryptosporidium spp. are protozoa that present important concerns for livestock production and public health (Meireles, 2010; Díaz *et al.*, 2018). These parasites belong to the phylum Apicomplexa, class Gregarinomorphea, and are frequently implicated in outbreaks of diarrhea worldwide (Cavalier-Smith, 2014; Clode *et al.*, 2015; Ryan *et al.*, 2016). They cause damage to the intestinal epithelium, leading to reduction in nutrient absorption, dehydration, and ultimately death of some animals (Thomson *et al.*, 2017; Abreu *et al.*, 2019). From an economic point of view, studies have demonstrated that the body weight gain in infected calves is 55% lower than in healthy animals (Abreu *et al.*, 2019), which compromises livestock production (Thomson *et al.*, 2017).

Cattle may be infected by several species of *Cryptosporidium*, but only some of these (e.g., *Cryptosporidium parvum*, *Cryptosporidium bovis*, *Cryptosporidium ryanae* and *Cryptosporidium andersoni*) are epidemiologically important. However, in calves, especially the species *C. parvum*, it is responsible for clinical infections, economic losses and public health implications (Gong *et al.*, 2017). Natural parasitism by *Cryptosporidium* spp. in Brazilian cattle has been detected throughout the country (Silva Junior *et al.*, 2011; Abreu *et al.*, 2019). Among the main factors favoring occurrences of cryptosporidiosis, age, immunological competence and concomitant rearing of cattle with other animal species are the most relevant (Ayele *et al.*, 2018).

The epidemiological chain of these protozoa evolves through both on and off-host factors. The main transmission route is by the ingestion of oocysts that are present in the environment, water and food (Wells *et al.*, 2019). It is important to note that because water sources (e.g., rivers and lakes) are close to farms, inappropriate discarding of production waste may affect the water quality and consequently increase the risk of pathogen infection (Andrade *et al.*, 2018).

The assessment of risk factors is pivotal, as it helps to adopt prophylactic measures, consequently reducing the occurrence of *Cryptosporidium* spp. in calves (Díaz *et al.*, 2018). However, these factors are poorly investigated in many regions despite the fact that it has been considered an important cause of neonatal diarrhea and of consequent economic losses in dairy farms (Ayele *et al.*, 2018). In the state of Pernambuco, there are records of this infection only in sheep (Tembue *et al.*, 2006) and goats (Souza *et al.*, 2015). The Agreste region of Pernambuco is the most important milk-producing area in this state. Hence, specific studies focusing on the detection of *Cryptosporidium* spp. in calves in this region are needed. The aim of this study was to determine the prevalence and risk factors associated with infection by *Cryptosporidium* spp. in calves from Northeastern Brazil.

MATERIAL AND METHODS

This study was conducted in 35 calve farms (up to 11 samples from each farm) of different breeds (Dutch and Crossbreed Dutch), averaging milk production of 900L/day each, located in the Southern Agreste Microregion (8°53′25″ South and 36°29′34″ West) of the state of Pernambuco, Northeastern Brazil (Figure 1). This region is characterized by a warm dry sub-humid tropical climate, with an average annual temperature of 21 °C (ranging from 15 °C to 31 °C), average rainfall of 198 mm (ranging from 65 mm to 325 mm) and relative air humidity of 90% (ranging from 80% to 98%). The Ethics Committee for Animal Use (ECAU) of the Federal Rural University of Pernambuco approved all procedures performed in this study (approval number: 73/2018).

Between April 2018 and April 2019, fecal (10 g) samples (n = 385) were collected from the rectum of animals (up to 10 months of age and raised in collective calve facilities) using plastic gloves and were maintained at 8 °C for three hours without preservatives until laboratory processing. The samples were divided into three groups according to the age of the animals [G1: up to 30 days old (n = 111); G2: from 31 to 60 days old (n = 124); and G3: from 61 days to 10 months old (n = 150)]. At the time of sample collection, each animal was physically examined, and the diarrheic condition determined according to Hulsen (2016). Afterwards, an epidemiological questionnaire (Supplementary file 1) seeking information about the hygiene conditions and characteristics of the herd was applied to the farm owner.

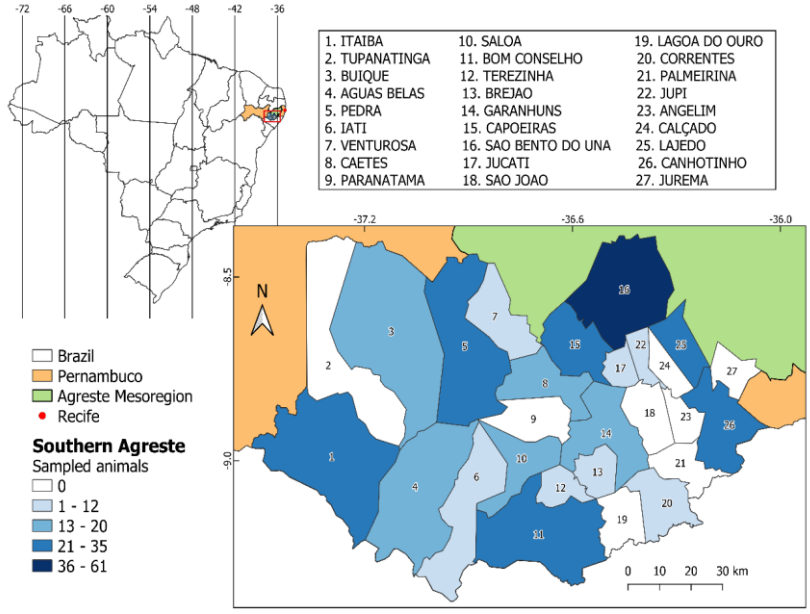

Figure 1. Number of animals sampled on the Southern Agreste Microregion of Pernambuco, Brazil.

The minimum sample size was calculated considering a cattle population of 510,824 (Municipalities, 2016), estimated prevalence of 50% for *Cryptosporidium* spp. infection, 95% confidence level and statistical error of 5% (Thrusfield, 2004). The farms were randomly selected by convenience (Reis, 2003). The samples were processed to identify *Cryptosporidium* spp. through the centrifugal sedimentation method in formaldehyde (Ritchie, 1948; David *et al.*, 1989), followed by the modified Ziehl-Neelsen staining technique (Henriksen and Pohlenz, 1981). The identification of oocysts was based on morphometrical features and they were measured using the AxioVision software (release 4.8). The entire length of the slide (duplicate) was analyzed by optical microscope using objectives of 40x and 100x.

The Lilliefors test was used to ascertain whether the data presented normal distribution. The Yates-corrected chi-square test (χ^2) was used to compare positive results for *Cryptosporidium* spp. according to the age of the calves and presence of clinical signs. The significance level was set at 5%. SPSS version 13.0 was used to perform all analyses except for risk factors using the *Odds Ratio* (OR), which were calculated using the EPI-INFO™ 7.2.2.6 software. The Quantum geographic information system (QGIS 3.8.0 Zanzibar) was used to process a choropleth vector map. Subtitle classes were created through Jenks optimization (Jenks and Caspall, 1971).

RESULTS

Out of all the samples analyzed, 25.7% (99/385) scored positive for the presence of *Cryptosporidium* spp. oocysts. The highest prevalence was detected among the animals of G2 (from 31 to 60 days old), but without any statistical difference (χ^2 = 1.6400; p = 0.440436). It is important to note that diarrheal disease was observed in 28.3% (28/99) of the positive animals, while the other 71.7% (71/99) did not present any clinical signs (χ^2 = 2.0447; p = 0.152733). Conversely, among negative animals, 20.6% (59/286) exhibited diarrhea whereas 79.4% (227/286) did not present clinical signs (Table 1). Oocysts presented a mean length of 3.98 ± 0.69µm and mean width of 4.46 ± 0.71µm (Figure 2).

The following parameters (Table 2) were considered risk factors: contact with other species (i.e., goat and sheep) (OR = 3.33; p = 0.0000), use of a semi-intensive rearing system (OR = 1.70; p = 0.024) and the poor hygienic sanitary conditions (i.e., fecal contamination of food and water) (OR = 1.64; p = 0.029).

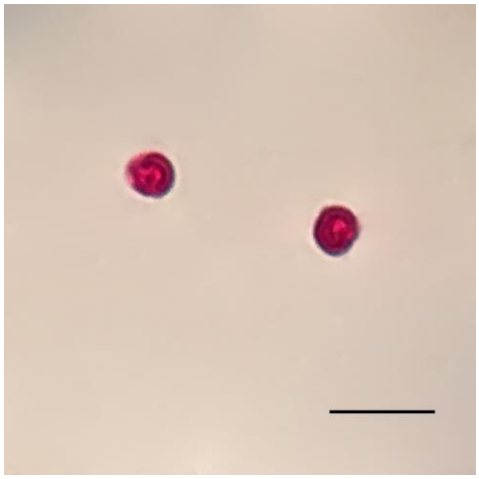

Figure 2. *Cryptosporidium* spp. oocysts stained with modified Ziehl-Neelsen staining technique (scale-bar = 10µm).

Table 1. Prevalence of *Cryptosporidium* spp. according to the age range and presence or absence of diarrhea in the state of Pernambuco, Brazil

Age (days)	Positive (n)	With Diarrhea		No Diarrhea		Negative (n)	With Diarrhea		No Diarrhea	
		AF (RF %)	95% CI	AF (RF %)	95% CI		AF (RF %)	95% CI	AF (RF %)	95% CI
0–30	26	11 (42.1)	23.3-61.3	15 (57.7)	38.8-76.6	85	30 (35.3)	25.2-45.4	55 (64.7)	54.6-74.8
31–60	37	13 (35.1)	20.1-50.1	24 (64.9)	49.6-80.2	87	22 (25.3)	16.2-34.4	65 (74.7)	65.6-83.8
>60	36	4 (11.1)	1.1-21.1	32 (88.9)	78.7-99.1	114	7 (6.1)	1.7-10.5	107 (93.8)	89.5-98.3
Total	99	28 (28.3)	19.5-37.1	71 (71.7)	62.9-80.5	286	59 (20.6)	16.0-25.2	227 (79.4)	74.8-84.0

Table 2. Univariate analysis of risk factors associated with *Cryptosporidium* spp. in calves of the state of Pernambuco, Brazil

Variable	N	Modified Ziehl-Neelsen Positive n (%)	Univariate Analysis OR (95% CI)	*p*-value
Age (days)				
0-30	111	26 (23.4)		
31-60	124	37 (29.8)	1.4 (0.7 - 2.6)	0.168
> 60	150	36 (24.0)	0.7 (0.4 - 1.3)	0.170
Species in contact				
Bovine	114	49 (42.98)		
Bovine, goat and sheep	271	50 (18.5)	3.3 (1.9 - 5.5)	0.000*
Breeding System				
Intensive	251	73 (29.1)		
Semi intensive	134	26 (19.4)	1.7 (1.0 - 2.9)	0.024*
Hygiene of the premises				
Yes	240	70 (29.2)		
No	145	29 (20.0)	1.6 (0.9 - 2.8)	0.029*
Presence of diarrhea				
Yes	87	28 (32.2)		
No	298	71 (23.8)	1.5 (0.8 - 2.6)	0.077

DISCUSSION

This study demonstrated that *Cryptosporidium* spp. is a sanitary threat to calves reared in a milk production region of Northeastern Brazil. The overall prevalence mean observed (i.e., 25.7%) is higher than others studies previously reported in different Brazilian regions such as 10.2% (Toledo *et al.*, 2017), 16.4% (Abreu *et al.*, 2019) and 17.1% (Rodrigues *et al.*, 2016). Most likely these differences occurred due to factors such as sample size, age of animals and diagnostic technique employed.

Although it has been demonstrated that this kind of parasitism especially affects young animals, no difference was observed among groups (G1, G2 and G3). In fact, the increase of age is a self-limiting factor in *C. parvum* infections, but in parasitism by other (host-adapted) *Cryptosporidium* species the subclinical condition is predominant in older animals (Thomson *et al.*, 2017; Åberg *et al.*, 2020). Nonetheless, it is important to note that infected animals, either young or adults, are important because they contaminate the environment, since they eliminate oocysts through their feces (Santos *et al.*, 2016). For a long time, the excretion of oocysts was frequently related to the presence of clinical signs such as diarrhea (Silva Junior *et al.*, 2011; Santos *et al.*, 2016). However, our data indicates that asymptomatic animals also eliminate oocysts; therefore, they may play an important role in contaminating the environment (Thomson *et al.*, 2017; Razakandrainibe *et al.*, 2018).

Several risk factors (e.g., age and herd size) have been related to infection by *Cryptosporidium* spp. (Ayele *et al.*, 2018; Manyazewal *et al.*, 2018). In this study, contact with other species (goat and sheep), use of a semi-intensive rearing system and absence of hygienic conditions (fecal contamination of food and water) were considered risk factors. This protozoan (e.g., *C. parvum*) may infect different vertebrate hosts and close contact with different animal species may increase in up to three times the risk of infection (Xiao and Fayer, 2008). At the same time, the rearing system is one of the most common risk factors, since close contact between susceptible and infected animals favors the dissemination of parasites, including *Cryptosporidium* spp. (Manyazewal *et al.*, 2018). All risk factors mentioned above contribute to the increase of *Cryptosporidium* oocysts in the environment. Hence, animals living in these conditions are in constant contact with these parasites facilitating their infection.

Undoubtedly, the absence of hygienic conditions is one of the most important causes of *Cryptosporidium* infection and this has frequently been reported in previous studies (Silva Junior *et al.*, 2011). The presence of organic matter such as feces provides an environment with adequate temperature and humidity for maintaining the oocysts, thus facilitating transmission. It is important to note that these protozoa may remain infective for more than six months in adequate environmental conditions (Alum *et al.*, 2014).

CONCLUSION

In conclusion, the data presented here shows that it is important to adopt hygiene measures (avoid fecal contamination of food and water) to control infection by *Cryptosporidium* spp., as well as to reduce the potential economic impact and prevent human infection. Additionally, it is important to highlight that this was the first epidemiological study to determine the prevalence of infection by these protozoa and the associated risk factors among calves in the state of Pernambuco, Northeastern Brazil.

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