Acta Veterinaria Brasilica March 13 (2019) 24-29

Acta Veterinaria Brasilica

Journal homepage: https://periodicos.ufersa.edu.br/index.php/acta/index

Original Article

Occurrence of *Cryptosporidium* spp. and its association with ponderal development and diarrhea episodes in nellore mixed breed cattle

Ocorrência de *Cryptosporidium* spp. e sua associação com o desenvolvimento ponderal e episódios diarreicos em bovinos mestiços da raça Nelore

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A R T I C L E I N F O

Article history
Received 19 September 2018
Received in revised form 12 December 2018
Accepted 17 December 2018

Keywords:
Cryptosporidiosis
Weight gain
Beef cattle
Diarrhea

A B S T R A C T

Due to the increasing importance of cryptosporidiosis in animal productivity, this study was carried out to investigate the occurrence of *Cryptosporidium* spp. and its association with animal weight development and fecal consistency in crossbred Nellore cattle. Thus, fecal samples of 30 cattle were collected biweekly, and their growth was accompanied by weighing animals from birth to 210 days of age (June 2014 to May 2015). The modified Ritchie and Ziehl-Neelsen techniques were used to screen for the presence of *Cryptosporidium* spp. oocysts. The chi-square, Fisher exact, Tukey and Spearman's correlation tests were used for statistical analysis. Oocysts of *Cryptosporidium* spp. were found in 69 (16.43%) of the 420 samples of cattle feces, and these parasitized animals presented a mean weight gain (4.76 kg) lower than that of non-parasitized individuals (10.58 kg) ($p<0.05$). The presence of this protozoan was detected in diarrhea and pasty stools (81.78%), indicating an association of the parasite with persistent diarrheal episodes ($p<0.001$). Among the bovines examined in this study, a higher occurrence of *Cryptosporidium* spp. was observed in lean diarrheic calves, which presented lower weight gain and poor productive performance.

Palavras-chave:
Criptosporidiose
Ganho de peso
Gado de corte
Diarreia

R E S U M O

Devido à crescente importância da criptosporidiose na produtividade animal, este estudo foi realizado com o objetivo de investigar a ocorrência de *Cryptosporidium* spp., e sua associação com o desenvolvimento ponderal e a consistência fecal em bovinos mestiços da raça Nelore. Assim, foram coletadas quinzenalmente amostras de fezes de 30 bovinos, acompanhando seu crescimento, por meio de pesagens, do nascimento aos 210 dias de idade, no período de junho de 2014 a maio de 2015. Para verificar a presença de oocistos de *Cryptosporidium* spp., foram realizadas as técnicas de Ritchie e Ziehl-Neelsen modificada. Para análise estatística, foram utilizados os testes qui-quadrado, exato de Fisher, Tukey e correlação Spearman. Dentre 420 amostras de fezes dos bovinos, em 69 (16,43%) foram encontrados oocistos *Cryptosporidium* spp., e estes animais parasitados apresentaram uma média de ganho de peso de 4,76 Kg, inferior aos animais não parasitados ($p<0,05$), que obtiveram uma média de ganho de peso de 10,58 Kg. A presença deste protozoário foi detectada em fezes diarreicas e pastosas (81,78%), indicando uma associação da presença do parasito com episódios diarreicos persistentes ($p<0,001$). Nos bovinos examinados neste estudo, foi observada maior ocorrência de *Cryptosporidium* spp., em bovinos magros, com diarreia, que apresentaram menor ganho de peso e baixo desempenho produtivo.

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http://dx.doi.org/10.21708/avb.2019.13.1.7977

INTRODUCTION

Beef cattle ranching is an economic activity of fundamental importance for Brazil. Among the sanitary problems, parasitoses act directly lowering sector indexes, directly reflecting animal production. Protozoans of the genus *Cryptosporidium* spp. have been highlighted as the cause of animal losses, such as decreases in body weight gain, compromising ponderal development, and may lead animals to death. Li et al. (2013) diagnosed the following clinical signs: watery diarrhea, dehydration, and weight loss in calves challenged by a protozoan of the genus *Cryptosporidium* spp.

Cryptosporidiosis is a parasitosis caused by a protozoan of the genus *Cryptosporidium* spp., which recently became part of the class Gregarinomorphea, as a new order and subclass of Gregarines. It is an extra-cytoplasmic parasite of epithelial cells of gastrointestinal tract, and bovines are the main hosts for the species *Cryptosporidium parvum*, which presents a zoonotic potential, and may even affect humans, while the other species and genotypes are more specific but sporadic (THOMPSON; ASH, 2016). Its main transmission route is fecal-oral, where the host ingests oocysts from water or contaminated food (BALDURSSON; KARANIS, 2011).

This disease has as main consequences inflammation and atrophy of intestinal villi, resulting in absorption surface loss, nutrient transport imbalance (THOMPSON; PALMER; O'HANDLEY, 2008), and hence animal production impairment (DECUBELLIS; GRAHAM, 2013). In cattle, the highest excretion rate of *Cryptosporidium* spp. oocysts was observed in calves in the first days of age (FEITOSA et al., 2008).

Among domestic animals, it occurs more frequently in cattle, especially in animals in the growing phase, whose morbidity cases by *C. parvum* varies from 10 to 85%, causing episodes of diarrhea and anorexia, resulting in low growth rates. These low growth rates are expressive when there is an association with other infectious agents, or in animals with deficient nutritional status and compliant immunosuppression (THOMAS et al., 2007).

Due to the increasing importance of cryptosporidiosis as a zoonotic parasitic infection and its association with animal productivity impairment, the objective of this study was to investigate the occurrence of *Cryptosporidium* spp. and its association with ponderal development in crossbred Nellore cattle, reared in the municipality of Alvorada do Gurguéia, Piauí state, Brazil.

MATERIAL AND METHODS

Animals, study location, and ethical aspects

The present work was developed at the experimental farm of Alvorada do Gurgueia, which belongs to the Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas (CPCE) (08°22'39'' South and 43°51'34'' West), located in the municipality of Alvorada do Gurguéia, in the Sudoeste Piauiense mesoregion. This study was approved by the animal experiment ethics committee of the UFPI, under protocol number 034/11.

Data collection and experimental group selection

Data were collected from June 2014 to May 2015. A total of 14 samples were collected, eight in the dry period and six in the rainy season, in a 15-day interval between collections, totaling 420 samples. Seasonal variation between dry and rainy periods was set by the data from the meteorological station (INMET, 2018).

In this study, 30 crossbred Nellore cattle from a single herd were used, all born from multiparous cows. Data were collected from birth to 210 days of age. Prior to live-weight assessment, the animals were fasted at least 12 hours, in order to empty the digestive tract.

Data such as sex, age, weight, and stool consistency were observed and recorded in individual records at the time of collection. The latter variable was characterized into three classes: normal, pasty, and diarrheal. Nutritional status was also estimated through phenotypic observations of animal carcass conformation, attributing qualitative classifications as lean, medium, and fat, besides factors predisposing to infection.

Individual stool specimens were collected directly from the rectal bulb of animals, using plastic bags to avoid contamination with free-living larvae or other pathogens presumably in the environment. After collection, the specimens were identified and conditioned in an isothermal box at 4 °C. Simultaneously, the animals were weighed for ponderal development monitoring, during the 210 days of age, aiming to associate growth rates with parasitic infections.

The breeding system used in the farm was semi-intensive, with herds spending the day grazing in 50-hectare pastures, formed by *Brachiaria decumbens* mixed with Andropogon grass (*Andropogon gayanus* cultivar Planaltina). In the late afternoon, the animals were allocated in collective stalls with dirt floors, in which they were given concentrate supplementation containing 40% maize meal, 15% soybean meal, 10% urea, 30% common salt, and 5% calcitic limestone, as well as mineral supplementation and water *ad libitum*

Fecal parasitological analysis

Samples were maintained at a temperature of 4°C until processing. The concentration of *Cryptosporidium* spp. oocysts was measured by the modified technique of Ritchie (1948), and the staining of slides was made in duplicate by the modified Ziehl-Neelsen technique (HENRICKSEN; POHLENZ, 1981).

Statistical analysis

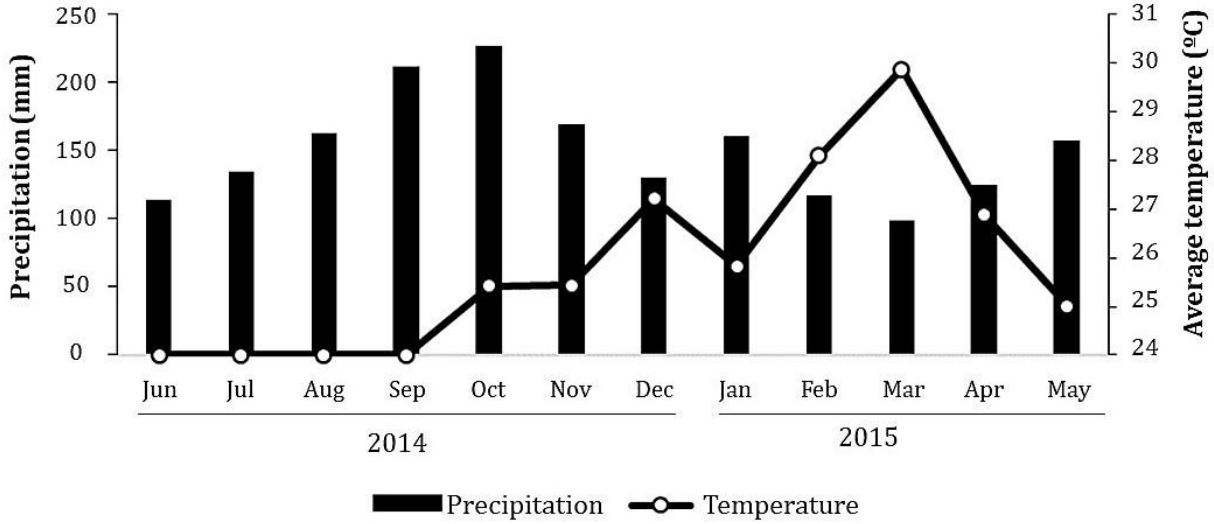
The results were submitted to the analysis of variance (ANOVA) by nonparametric statistics using PROC NPAR1WAY procedure (SAS, 1999). The procedures PROC FREQ [chi-square test (χ^2) or Fisher's exact test] and PROC CORR (Spearman correlation analysis) were used to verify respectively frequency and association between *Cryptosporidium* spp. occurrence in non-parametric data, for each age group evaluated, regarding sex, nutritional status, fecal consistency, collection season, and contemporary group. Lastly, weight gain was assessed using PROCGLM procedure and Tukey's test at 5% probability.

RESULTS AND DISCUSSION

Of the 420 fecal samples of bovines, 69 (16.43%) had oocysts of *Cryptosporidium* spp. A similar result was observed by Rodrigues et al. (2016), working under the same conditions, these authors diagnosed parasites from this genus in 17.1% (26/152) of the fecal samples taken from cattle up to three months of age. Likewise, Silva Júnior et al. (2011) found oocysts in 21.62% (77/356) of the calf stool samples collected from ten farms in the south of Minas Gerais. By contrast, Lima et al. (2013), using molecular characterization, observed these parasites in only 5.3% (16/300) of the parasitized calves reared in the city of Formiga (Minas Gerais), which is smaller than that found in this study.

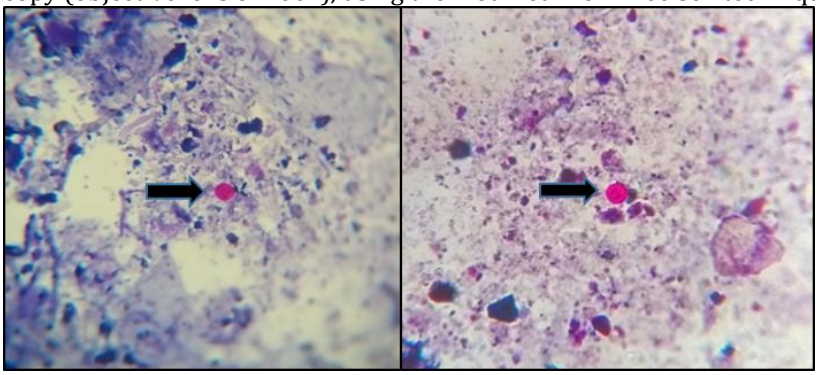
Figure 1 shows the climate data for the study location and period, including environmental temperature and monthly precipitation. During the experimental period, a clear dry season was observed from May to November and another rainy one from December to April. These data were gathered from the website of the meteorological station belonging to the National Meteorological Institute (INMET, 2018) (Figure 1).

Figure 1 – Monthly averages of temperature and precipitation from June 2014 to May 2015 in Alvorada do Gurguéia, Piauí, Brazil.



Cryptosporidium spp. oocysts were evidenced when stained in purple by carbolic fuchsin, and the other structures in blue (Figure 2). Huber; Bomfim; Gomes (2003) reported that oocysts can be more easily identified by the Ziehl-Neelsen technique, even in samples with a low number of them, since color contrast allows easy visualization with no fecal debris interference in diagnosis.

Figure 2 – Microscopic appearance of *Cryptosporidium* spp. oocysts in fecal smears of crossbred Nellore calves (arrows) diagnosed by light microscopy (objective lens of 100x), using the modified Ziehl-Neelsen technique.



Artificial drinking and feeding troughs are predisposing factors for infection with *Cryptosporidium* spp. since oocysts may remain viable for a long term therein. This is because these environments have propitious humidity and temperature conditions (such as in the study site) for the maintenance of the protozoan lifecycle, and animals could be infected by fecal or nasal shedding (ALMEIDA; OLIVEIRA; TEXEIRA, 2008). This might have been the route through which the parasite infected the animals in this study. Silva Júnior et al. (2011) argued that early exposure of calves to water and feed in the first days of life may directly affect infection rates since both can be contaminated with oocysts.

Parasitized animals had a lower weight gain (4.76 kg) if compared to the non-parasitized ones (10.58 kg) ($p < 0.05$). Animals not parasitized by *Cryptosporidium* spp. presented a 55% higher weight gain than the parasitized bovines. According to Vieira et al. (1997), Soltane et al. (2007), Wang et al. (2011), Noordeen et al. (2012), the protozoan can cause direct and indirect economic losses in production, as it jeopardizes the development and production of animals. The reduction in live-weight gain of the parasitized individuals, especially when kept in a breeding system with sanitary and nutritional shortcomings, generate economic losses to breeders (LI et al., 2013).

In this study, non-parasitized animals had a daily mean weight gain of 0.705 kg, similar to the finding of Cappelle et al. (2001) of 0.740 kg. However, our finding was superior to that obtained by Perotto et al. (2001), who observed a weight gain of only 0.339 kg. Such variation may be due to the rearing conditions and climatic differences between the regions.

We also found oocysts to be more frequent in diarrheal feces (55.55%) followed by pasty stools (26.23), indicating that this infection is associated with diarrheal episodes ($p < 0.001$) (Table 1). Similar results were described by Pulido-Medellín et al. (2014), who observed that 70.4% of the animals with diarrhea had oocyst excretions. According to Oliveira Filho et al. (2007) and Chako et al. (2010), diarrhea is a clinical sign related to infection by this enteropathogen.

Table 1 – Number of fecal samples, total number and percentage of positive samples for *Cryptosporidium* spp., fecal consistency and nutritional status of Nellore cattle, diagnosed by modified Ritchie and Ziehl-Neelsen techniques.

Stool Consistency **	Total stool sample (nº)	*Cryptosporidium* spp.	
		Positive sample	%
Normal/consistent	287	13	04.52
Pasty	61	16	26.23
Diarrhea	72	40	55.55
Nutritional Status **			
Lean	22	9	41.00
Medium	317	48	15.14
Fat	81	12	14.81

** Significant by the χ^2 test at 0.1% probability ($p < 0.001$).

Safavi et al. (2011) found 36.6% (82/224) of fecal samples from calves to be positive for *Cryptosporidium* spp. These infected animals were less than one month old and, of these, 51.8% of them had diarrhea. The authors also highlighted that the highest infection rates were diagnosed in calves between 8 and 14 days of age. In short, these findings reveal a higher infection risk within the second week of life and that the peak oocyst excretion in calves with diarrhea is six times larger than that in non-diarrheic animals. Likewise, Asadpour et al. (2013) observed by microscopy that 28.3% (85/300) of calf fecal samples were positive for *Cryptosporidium* spp., and when analyzing fecal consistency, they found the all animals presenting positive samples were diarrheic.

Oliveira Filho et al. (2007) pointed out the protozoan *Cryptosporidium* spp. as one of the most worrisome enteropathogens of domestic ruminants. This organism leads to malabsorption syndrome, impairing food digestion and causing host diarrhea, as well as atrophy of microvilli and loss of digestive enzymes (LI et al., 2013).

Feitosa et al. (2008) evaluated 57 positive animals for *Cryptosporidium* spp. and observed that 26.3% had or developed diarrhea during clinical follow-up, while 73.7% of the animals were asymptomatic carriers, demonstrating their importance as a primary source of environmental contamination and Cryptosporidium infection.

Nutritional status was compared between animals with and without parasite presence. Among the parasitized animals, 41% showed a poor nutritional status, 15.14% a medium nutritional status, and 14.81% were fat, showing a larger parasitic infection in lean animals ($p < 0.001$). This outcome might have occurred due to the negative influence of the parasite on the gastrointestinal tract of animals (SILVERLÂS et al., 2010). Conversely, Lima et al. (2013) diagnosed the occurrence of *Cryptosporidium* spp. infection, but found no significant effect when comparing positive animals and their body scores.

Several factors can affect animal nutritional status (such as weight development and gain). Among these factors are animal management type, climatic conditions, forage availability, and parasitic diseases (e.g., cryptosporidiosis). As previously reported, the action of

these parasites causes food malabsorption, which can slow down growth, increase weight loss and, in some cases, lead to death (RIEUX et al., 2013; VARGAS JÚNIOR et al., 2014).

The occurrence of *Cryptosporidium* spp. was higher in younger animals, aged 01 to 90 days, among which a total of 21.66% (180) of positive samples were found. Moreover, the percentage of positive samples decreased ($p < 0.05$) as animal age increased (Table 2). In young calves up to three months of age, cryptosporidiosis can lead to severe diarrhea and cause morbidity and even death (CHAKO et al., 2010).

Table 2 – Total number of studied samples, positive samples for *Cryptosporidium* spp., and the relationship among age, sex, and sampling station of Nellore cattle diagnosed by modified Ritchie and Ziehl-Neelsen techniques.

Age of the 30 studied animals in different seasons*	Total stool sample (nº)	*Cryptosporidium* spp.	
		Positive sample	%
01-90 days	180	39	21.66
91-150 days	120	18	15.00
151-210 days	120	12	10.00
Sex ns			
Male	322	50	15.52
Female	98	19	19.38
Collection season ns			
Dry	232	45	19.40
Rainy	188	24	12.76

*Significant by the χ^2 test at 5% probability ($p<0.05$). ns – non-significant by the χ^2 test.

The highest rate of oocyst excretion was observed in calves aged between 8 and 14 days (14.5%), and the lowest (6.4%) in older animals (22 to 30 days of life). However, animals started to excrete oocysts within 24 hours, which is a strong indication of animal infection soon after birth. Oocysts might have been transmitted through water and contact with contaminated feces (FEITOSA et al., 2008).

Higher prevalence of Cryptosporidium infection was detected in fecal samples from cattle up to three months of age (RODRIGUES et al., 2016). However, no difference was observed in infection among three age groups of calves, which varied from birth to six months of age (DESTRO et al., 2014). The highest intensity of parasitic infection was observed in animals up to seven days old, indicating that this age group is, from an epidemiological point of view, the most susceptible to infection (DEL COCO; CÓRDOBA; BASUALDO, 2008).

When comparing parasite presence and sex of host, no difference was observed ($p > 0.05$), corroborating the findings of Rodrigues et al. (2016), Almeida; Oliveira; Texeira (2008) and Almeida et al. (2010), who stated that *Cryptosporidium* spp. infection occurs regardless of host sex. No difference was observed between parasitized animals within the same collection season ($p > 0.05$). Silva Júnior et al. (2011) observed a higher occurrence of *Cryptosporidium* positive calves during the rainy season (48/131).

Table 3 displays the correlations among the variables host sex, collection season (EC), contemporary group (CG), *Cryptosporidium* spp. presence, stool consistency, and animal nutritional status. A positive correlation ($p < 0.05$) was observed between *Cryptosporidium* spp. infection and stool consistency, with a correlation coefficient of 0.50. This justifies the fact that pasty and diarrhea feces showed a higher presence of oocysts.

Table 3 – Spearman correlation coefficients among the evaluated variables in crossbred Nellore cattle from birth to 210 days of age.

	EC	GC	*Cryptosporidium*	feces	EN
Sex	-0.02	0.20*	0.04	0.01	-0.05
EC	-	-0.25*	0.08	0.10	0.01
GC		-	0.01	-0.05	-0.02
Cryptosporidium			-	0.50*	-0.08
feces				-	-0.10

Collection season (EC), contemporary group (GC), and nutritional status (EN). *Significant correlation ($p <0.05$).

Diarrhea remains as one of the leading causes of morbidity and mortality in calves (CHAKO et al., 2010) since it is associated with dehydration, weakness, and eventual death. Vargas Júnior et al. (2014) warned of the importance of cryptosporidiosis as a primary agent of diarrhea in calves. Regarding management, preventive measures are needed to reduce economic losses, environmental contamination, and risks to public health.

Thus, it is extremely important to obtain data on the occurrence and distribution of *Cryptosporidium* spp. by region, as well as to gain knowledge about its seasonal occurrence, so that its epidemiology can be defined for future planning and establishment of preventive measures.

CONCLUSION

Among the bovines examined in this study, the occurrence of *Cryptosporidium* spp. was higher in lean diarrheic calves, which presented lower weight gain and poor productive performance.

REFERENCES

ALMEIDA, A. J. et al. Risk factors associated with the occurrence of *Cryptosporidium parvum* Infection in calves. **Arquivo Brasileiro Medicina Veterinária e Zootecnia**, v. 62, n. 6, p. 1325-1330, 2010.

ALMEIDA, A. J.; OLIVEIRA, F. C. R.; TEXEIRA, C. S. Risco relativo da infecção por parasitos do gênero C*ryptosporidium* em bezerros bovinos no Norte do estado do Rio de Janeiro, Brasil. **Revista Brasileira de Parasitologia Veterinária**, v. 17, n. 1, p. 243-248, 2008.

ASADPOUR, M. et al. Prevalence and molecular identification of *Cryptosporidium* spp. in pre-weaned dairy calves in Mashhad Area, Khorasan Razavi Province, Iran. **Iranian Journal Parasitology**, v. 8, n. 4, p. 601-607, 2013.

BALDURSSON, S.; KARANIS, P. Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2004–2010. **Water Research**, v. 45, p. 6603-6614, 2011.

CAPPELLE, E. R. et al. Estimativas do Consumo e do Ganho de Peso de Bovinos, em Condições Brasileiras. **Revista Brasileira de Zootecnia**, v. 30, n. 6, p. 1857-1865, 2001.

CHAKO, C. Z. et al. Cryptosporidiosis in people: It's not just about the cows. **Journal of Veterinary Internal Medicine**, v. 24, n. 1, p. 37-43, 2010.

DECUBELLIS, J.; GRAHAM, J. Gastrointestinal disease in guinea pigs and rabbits. **Veterinary Clinics of North America: Exotic Animal Practice**, v. 16, n. 2, p. 421-435, 2013.

DEL COCO, V. F. D.; CÓRDOBA, M. A.; BASUALDO, J. A. *Cryptosporidium* infection in calves from a rural area of Buenos Aires, Argentina. **Veterinary Parasitology,** v. 158, n. 1-2, p. 31-35, 2008.

DESTRO, K. C. et al. Ocorrência de *Cryptosporidium* spp. em bezerros bubalinos no estado do Pará**. Arquivos do Instituto Biológico**, v. 81, n. 4, p. 368-371, 2014.

FEITOSA, F. L. et al. Importância de *Cryptosporidium* spp. como causa de diarreia em bezerros. **Pesquisa Veterinária Brasileira**. v. 28, p. 452-456, 2008.

HENRICKSEN, S.; POHLENZ, I. J. Staining of cryptosporidia by a modified Zielh-Neelsen technique. **Acta Veterinaria Scandinavica**, v. 22, n. 3-4, p. 594-596, 1981.

HUBER, F.; BOMFIM, T. C. B.; GOMES, R. S. Comparação da eficiência da técnica de sedimentação pelo formaldeido-éter e da técnica de centrifugo-flutuação modificada na detecção de cistos de *Giardia* sp. e oocistos de *Cryptosporidium* spp. em amostras fecais de bezerros. **Revista Brasileira de Parasitologia Veterinária**, v. 2, p. 135-137, 2003.

INMET. **Instituto Nacional de Meteorologia**. Available at: <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>. Accessed on: May 10, 2018.

LI, S. et al. Infection of cattle with *Cryptosporidium parvum*: mast cell accumulation in small intestine mucosa. **Veterinary Pathology**, v. 50, n. 5, p. 842-848, 2013.

LIMA, R. C. A. et al. Caracterização molecular de *Cryptosporidium* spp. em bezerros (*Bos taurus* e *Bos indicus*) no município de Formiga, Minas Gerais – Brasil. **Semina: Ciências Agrárias**, v. 34, n. 6, p. 3747-3754, 2013.

NOORDEEN, F. et al. *Cryptosporidium,* an important enteric pathogen in goats. A review. **Small Ruminant Research**, v. 106, n. 2-3, p. 77- 82, 2012.

OLIVEIRA FILHO, J. P. et al. Diarreia em bezerros da raça Nelore criados extensivamente: estudo clínico e etiológico. **Pesquisa Veterinária Brasileira**, v. 27, n. 10, p. 419-424, 2007.

PEROTTO, D. et al. Ganho de Peso da Desmama aos 12 Meses e Peso aos 12 Meses de Bovinos Nelore e Cruzas com Nelore. **Revista Brasileira de Zootecnia**, v. 30, n. 3, p. 730-735, 2001.

PULIDO-MEDELLÍN, M. O. et al. Prevalencia y posibles factores de riesgo en la excreción de ooquistes de *Cryptosporidium* spp. en bovinos de Boyacá, Colombia. **Revista Mexicana de Ciências Pecuárias**, v. 5, n. 3, p. 357-364, 2014.

RIEUX, A. et al. Molecular characterization of *Cryptosporidium* spp. in pre-weaned kids in a dairy goat farm in western France. **Veterinary Parasitology**, v. 192, n.1-3, p. 268-272, 2013.

RITCHIE, L. S. An ether sedimentation technique for routine stool examinations. Bulletin United States Army. **Medical Department**, v. 8, n. 4, p. 326, 1948.

RODRIGUES, R. D. et al. Comparação da eficiência das colorações de Ziehl- Neelsen Modificado e Safranina Modificada na detecção de oocistos de *Cryptosporidium s*pp. (Eucoccidiorida, Cryptosporidiidae) a partir de amostras fecais de bezerros de 0 a 3 meses. **Ciência Animal Brasileira**, v. 17, n. 1, p. 119-125, 2016.

SAFAVI, E. A. et al. Prevalence of *Cryptosporidium* spp. infection in some dairy herds of Mashhad (Iran) and its association with diarrhea in newborn calves. **Comparative Clinical Pathology**, v. 20, n. 2, p. 103-107, 2011.

SAS, Institute. **User's guide: statistics**. Version 8.1. Cary: NC, 1999.

SILVA JÚNIOR, F. A. et al. Fatores de risco associados à infecção por *Cryptosporidium* spp. e *Giardia duodenalis* em bovinos leiteiros na fase de cria e recria na mesorregião do Campo das Vertentes de Minas Gerais. **Pesquisa Veterinária Brasileira**, v. 31, n. 8, p. 690-696, 2011.

SILVERLÂS, C. et al. *Cryptosporidium* infection in herds with and without calf diarrhoeal problems. **Parasitology Research**. v. 107, n. 6, p. 1435-1444, 2010.

SOLTANE, R. et al. Prevalence of *Cryptosporidium* spp. (Eucoccidiorida: Cryptosporidiidae) in seven species of farm animals in Tunisia. **Parasite**, v. 14, n. 4, p. 335-338, 2007.

THOMAS, A. et al. Molecular identification of *Cryptosporidium* spp. from fecal samples of felines, canines and bovines in the state of São Paulo, Brazil. **Veterinary Parasitology**, v. 150, p. 291-296, 2007.

THOMPSON, R. C. A.; PALMER, C. S.; O´HANDLEY, R. The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. **Veterinary Journal**, v. 177, n. 1, p. 18-25, 2008.

THOMPSON R. C. A.; A. ASH. A. Molecular epidemiology of Giardia and Cryptosporidium infections. **Infection, Genetics and Evolution**, v. 40, n. 4, p. 315–323, 2016.

VARGAS JÚNIOR, S. F. et al. Surto de criptosporidiose em bezerros no Sul do Rio Grande do Su1. **Pesquisa Veterinária Brasileira**, v. 34, n. 8, p. 749-752, 2014.

VIEIRA, L. S. et al. Outbreak of cryptosporidiosis in dairy goats in Brazil. **Veterinary Record**, v. 140, n. 16, p. 427-428, 1997.

WANG, R. et al. *Cryptosporidium andersoni* is the predominant species in post-weaned and adult dairy cattle in China. **Parasitology International**, v. 60, n. 1, p. 1-4, 2011.