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Occurrence of Cryptosporidium in Anglonubian goats in the municipality of Teresina, state of Piauí, Brazil

Ocorrência de *Cryptosporidium* em cabras da raça Anglonubiana no município de Teresina, estado do Piauí, Brasil

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Abstract
This research aimed to investigate the occurrence of *Cryptosporidium* and correlate it with types of housing, feces consistency, and physiological parameters related to the reproductive status of Anglo-Nubian goats reared in the State of Piauí, Brazil. A total of 180 fecal samples were collected from 60 non-pregnant and lactating goats with a mean weight of 35 kg, a body condition score of 3.5, and a mean age of three years from an experimental herd at the Federal University of Piauí (UFPI). Oocysts of protozoa of the genus *Cryptosporidium* could be found in the studied animals using the modified Ziehl-Neelsen technique in fecal smears and the image analysis system to perform morphometry. Each independent variable in the quantitative and qualitative analyses, that is, weight, body condition score (BCS), physiological status (non-pregnant or lactating), feces consistency (normal, pasty, or diarrheal), and floor types (concrete and slatted), was tested with the dependent variable (positive samples, i.e., the presence of *Cryptosporidium* oocysts). Twenty-four out of the total number of fecal samples were considered positive for the presence of the protozoan, which means that 13.3% of the animals were parasitized. Moreover, 100% of the positive feces samples had normal consistency (firm) and all parasitized animals were reared in pens with a concrete floor. A statistical variation was observed in the BCS of parasitized animals compared to non-parasitized ones ($p > 0.0253$). The results showed that the occurrence of *Cryptosporidium* in experimental goats located in the municipality of Teresina, State of Piauí, Brazil, was considered low, requiring sanitary management measures to prevent infection in animals and humans. This is the first report of *Cryptosporidium* infection in goats in the State of Piauí.
Keywords: goats; cryptosporidiosis; Ziehl-Neelsen; zoonosis.

Resumo
O objetivo desta pesquisa foi investigar a ocorrência de *Crypstoporidium* e correlacionar com tipos de alojamento, consistência das fezes e parâmetros fisiológicos ligados ao estado reprodutivo de cabras da raça Anglonubiana criadas no estado do Piauí, Brasil. Foram utilizadas 180 amostras de fezes de 60 cabra, com peso médio de 35kg, escore de condição corporal de 3,5, com idade em média de três anos, e cabras vazias e lactantes, de um rebanho experimental da Universidade Federal do Piauí (UFPI). Utilizando-se a técnica de Ziehl-Neelsen modificada em esfregaço fecal e sistema de análise de imagens para a realização da morfometria, foi possível encontrar oocistos de protozoários do gênero *Crypstoporidium* nos animais estudados. Nas análises quantitativa e qualitativa, cada variável independente: peso, escore de condição corporal (ECC), estado fisiológico (vazia ou lactante), consistência das fezes (normal, pastosa ou diarreica) e tipos de piso (concreto e ripado), foi testada com a variável dependente (amostras positivas, ou seja, presença de oocistos de *Cryptosporidium*). Do total de amostras fecais analisadas, 24 delas foram consideradas positivas à presença do protozoário, o que significa que 13,3% dos animais estavam parasitados na ocasião da pesquisa. Foi observado que 100% das amostras de fezes positivas apresentaram consistência normal (firme) e que todos os animais parasitados eram criados em aprisco com piso de concreto. Houve uma variação estatística no ECC dos animais parasitados comparados aos não parasitados ($p > 0,0253$). Os resultados evidenciaram que a ocorrência de *Cryptosporidium* em caprinos experimentais localizados no município de Teresina, no estado do Piauí, foi considerada baixa, sendo necessária medidas de manejo sanitário para prevenir a infecção nos animais e no homem. Este é o primeiro relato da infecção por *Cryptosporidium* em cabras no estado do Piauí.
Palavras-chave: caprinos; criptosporidiose; *Ziehl-Neelsen*; zoonose.

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1. Introduction

The rearing of goats has shown great relevance in the economic and social development in the Northeast of Brazil. The goat species has a quick economic return due to its precocity in producing high biological value protein, such as meat and/or milk, and its short biological cycle.[1] The Anglo-Nubian breed, brought to Brazil to form herds with dual aptitude (meat and milk), is among the exotic goat breeds most reared in the Northeast of Brazil.[2] Animals of this breed are rustic and adaptable to the semi-arid climate, with morphological characteristics of the coat and loss of heat by cutaneous evaporation, but they have good zootechnical indices, especially for meat production, compared to native breeds reared in the region.[3]

Nevertheless, the difficulty in maintaining the health of the herd compromises its efficiency.[4] Although there is information about infections and resistance by endoparasites in Anglo-Nubian animals in the Northeast region of Brazil, further studies about the infection by protozoa of different genera and species are still required. *Cryptosporidium* is a protozoan with worldwide distribution that can affect several species of domestic and wild animals, in addition to humans, being relevant to public health because it has zoonotic potential.[5, 6, 7] However, the transmission is occasional, as the wide variety of species and genotypes are more specific.[8]

The genus *Cryptosporidium* has intracellular and extracytoplasmic locations, mainly in the cells of the epithelium of the gastrointestinal tract, causing important lesions in the microvilli of the organ, which compromises the process of nutrient absorption.[9] The main clinical signs that characterize this infection are watery and bloody diarrhea, apathy, and growth retardation, which can culminate in the death of affected individuals, whether humans or animals.[10] The main route of transmission of the disease is fecal-oral.[11]

Cryptosporidium spp. can infect goats in different age groups, being more common in newborn animals and/or up to nine months old,[12] males and females, and different breed patterns.[13] The first record of cryptosporidiosis in goats was in Australia in a young animal aged two weeks, which presented acute diarrhea before dying.[14] The goat species plays an important role in the transmission of cryptosporidiosis, as these animals are possible reservoirs of *Cryptosporidium parvum*, a species that has scientifically proven zoonotic potential.[15]

This disease is an infection that affects the gastrointestinal tract of animals, damaging the intestinal microvilli, and leads to a decrease in nutrient absorption, causing great damage to health and production indices, such as a reduction in weight gain and feed conversion of protein into high biological value feed, such as milk and meat.[16, 17] Some records of cryptosporidiosis in goats in the Northeast of Brazil were reported by Brito et al.[18] and Souza et al.[19] in the States of Ceará and Pernambuco, respectively.

Currently, there are no reports of infection by protozoa of the genus *Cryptosporidium* in the goat species in the State of Piauí. Therefore, this research aimed to investigate the occurrence of these parasites and correlate their presence with types of housing, feces consistency, and physiological parameters related to the reproductive status of Anglo-Nubian goats reared in the State of Piauí, Brazil.

2. Material and methods

2.1 Study site and animals

All experimental procedures involving animals were approved by the Ethics in Animal Use Committee (CEUA) of the Federal University of Piauí, Brazil (protocol number 259/16). Sixty purebred Anglo-Nubian goats were used in the experiment, 36 doeling and 24 multiparous animals, with ages ranging from one to seven years, belonging to the experimental herd of the Federal University of Piauí (UFPI), located in the city of Teresina. The municipality is located at the geographic coordinates 5°5′20″ S and 42°48′7″ W, with an altitude of 72 m.[20]

The pen consisted of two distinct facilities, one for doeling goats, built on the ground with a concrete floor and masonry structure, and the other for multiparous goats, with a suspended floor (slatted) made of wood. Both classes of animals had access to pasture in their paddocks. The animals were kept in a semi-confinement system, in which they remained overnight sheltered in the facilities, and released for eight hours a day on irrigated Tanzania grass (*Panicum maximum*) and brachiaria pastures, in addition to native pastures, such as sabiá (*Mimosa caesalpiniifolia* Benth). Water and mineral salt were provided ad libitum. Lactating females received commercial feed with 16% crude protein.

2.2 Collection of fecal samples

A total of 180 fecal samples were collected from 60 purebred Anglo-Nubian goats. The collections were carried out in February, April, and June 2017, totaling three samples collected per animal in each of these months. The same amounts of samples were collected from the same animals, that is, three samples were collected per animal. The samples, with an average weight of 5 g each, were collected directly from the rectal ampoule into plastic packages. All samples were identified and placed in a thermal container with ice for conservation and subsequent laboratory analysis. The different feces consistencies were observed at the time of collection of fecal samples and recorded on individual

cards, being classified as normal (firm), pasty, and/or diarrheal, in addition to information on the physiological state of the goats, classified as non-pregnant (neither lactating nor pregnant) or lactating, and the type of facility (slatted pen or concrete floor).

2.3 Laboratory analysis of fecal samples

The presence of oocysts of *Cryptosporidium* spp. in the collected fecal samples was verified by the modified Ritchie[21] and modified Ziehl-Neelsen[22] techniques for oocyst staining in fecal smears. The microscopic analysis was carried out with two slides per sample, which were observed under an optical microscope in a 100x objective (immersion). The morphometric analysis of the oocysts was performed using an AmScope® 3.7 MU1400-CK digital camera coupled to the objective lens of the microscope, which launches the images to a computer to perform the measurements using the AmScope® image analysis system, in which the diameter of each oocyst was measured in micrometers.

2.4 Data collection of weight and body condition score

Productive data related to weight gain, such as individual weighing and body condition score (BCS) were collected. The animals were weighed individually and scores from one to five were assigned for the BCS evaluation. Score one represents poor body condition, indicating that the spinous apophyses and transverse apophyses are easily felt on palpation. In contrast, score five represents excessive fat deposition, which makes it impossible to palpate the apophyses. Intervals of 0.5 points were analyzed in the score (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0).[23]

2.5 Statistical evaluation of data

The statistical data analysis was performed univariately using the SAS[24] statistical package, using the procedures PROC CORR and PROC GLM. In the quantitative and qualitative analyses, each independent variable, that is, weight, body condition score (BCS), physiological status (non-pregnant or lactating), feces consistency (normal, pasty, or diarrheal), and floor types (concrete and slatted), was tested with the dependent variable (samples positive for the presence of *Cryptosporidium* spp. oocysts). The significance level established in the analysis was 5% by Tukey's test.

3. Results

This is the first report of infection by *Cryptosporidium* spp. in the goat species in the State of Piauí. Twenty-four (13.33%) out of the total of 180 samples had *Cryptosporidium* spp. oocysts. Each of the 48 slides contained from one to four oocysts (Figure 1). All oocysts were measured after identification and presented measurements ranging from 4.82 to 6.51 µm in diameter.

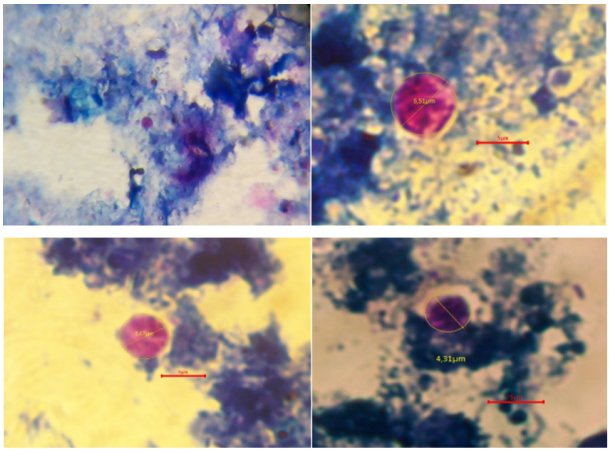

Figure 1. Oocysts of protozoa of the genus *Cryptosporidium* in fecal smears from Anglo-Nubian goats in the municipality of Teresina, State of Piauí, Brazil.

None of the positive samples were from animals that had diarrhea during the collection period, that is, 100% of the positive fecal samples had a normal consistency. Only two fecal samples had a pasty consistency, both negative for the protozoan presence (Table 1). Therefore, no correlation was found between the presence of *Cryptosporidium* and feces consistency.

Table 1. Relationship between feces consistency and the occurrence of protozoa of the genus *Cryptosporidium* in fecal samples from Anglo-Nubian goats in the municipality of Teresina, State of Piauí, Brazil

Sample	Feces consistency		
	Normal	Pasty	Diarrheal
Positive	24	00	00
Negative	154	02	00

The presence of *Cryptosporidium* spp. in the feces of the evaluated animals was correlated to two different types of flooring in the facilities where the animals were housed (Table 2). All parasitized animals were reared in pens with a concrete floor.

Table 2. Influence of two different types of flooring (concrete and slatted) on the occurrence of goats infected by protozoa of the genus *Cryptosporidium* in the municipality of Teresina, State of Piauí, Brazil

Type of floor	Total animals/ positive animals	Occurrence (%)	*p-value
Concrete floor	30/8[a]	13.33	0.0019
Suspended floor	30/0[b]	0	

The comparison between the influence of the physiological state, weight, and BCS of goats and the

presence of *Cryptosporidium* spp. showed that all animals positive for the infection were non-pregnant, with no significant difference between the mean weights when comparing parasitized and non-parasitized animals. A small statistical difference was observed between the BCS of parasitized and non-parasitized animals (Table 3).

Table 3. Influence of physiological status, weight, and body condition score (BCS) in Anglo-Nubian goats with and without infection by protozoa of the genus *Cryptosporidium* in the municipality of Teresina, State of Piauí, Brazil.

Parameter		Total animals/ positive animals	*p-value
Physiological status	Non-pregnant	45/8[a]	0.0819
	Lactating	15/0[a]	
Weight	Parasitized	36.10[a] kg	0.6191
	Non-parasitized	34.00[a] kg	
BCS	Parasitized	3.87[a]	0.0253
	Non-parasitized	3.15[b]	

*Probability <0.005% by Tukey's test.

4. Discussion

The occurrence of *Cryptosporidium* spp. was also reported in sheep in the State of Piauí[25] in fecal samples from 30 females, with oocysts of the protozoan being found in eight samples (8/30). Sixty-nine (16.43%) out of 420 fecal samples from the bovine species[26] also had oocysts of *Cryptosporidium* spp. The occurrence of *Cryptosporidium* in the municipality of Teresina was also reported in sheep[25] and cattle in Piauí.[26] The world's mean of infection by oocysts of this protozoan in goats ranged from below 5% and above 35%, with a mean of 15%.[27]

The morphometry of the oocysts found in this study allowed identifying the species of *Cryptosporidium*. In the literature, *C. andersoni*, *C. parvum*, and *C. bovis* are mentioned as the species commonly found in domestic ruminants.[28] All infected animals were over one year old, which is not common to observe, as the infection is commonly reported in animals younger than five months old[18] and in individuals up to 9 months old.[12] The animals evaluated in this study may be parasitized because they are already adults, and the non-manifestation of the disease may be related to their resistance, as the production of antibodies that block the pathogenic activity of the protozoa is in the pre-patent period when there is no elimination of oocysts in the feces.

An investigation of the presence of *Cryptosporidium* in goats less than one year old (241 to 360 days old) in the State of Ceará showed no parasitized animals.[18] *Cryptosporidium* oocysts were found in only 0.50% of 207 fecal samples from adult goats in a study carried out in India.[29] A study conducted in Spain found 6.40% (171 animals) of positive feces samples in adult goats,[30] corroborating a study carried out in Papua New Guinea, in which 4.40% of 228 animal samples were positive.[31] In both studies, the age group assessed was similar to that of our research.

Diarrhea is one of the most evident clinical signs in an animal parasitized by *Cryptosporidium*.[10] This symptom was only observed in young goats parasitized by this protozoan, mainly in animals up to nine months old,[32] but the animals evaluated in this study were older than one year and those parasitized did not have diarrhea.

A study conducted with young goats in the municipality of Quixadá in the State of Ceará showed that the most frequent *Cryptosporidium* species were *C. xiaoi* and *C. ubiquitum*, the former being found in all evaluated samples, while *C. ubiquitum* and *C. meleagridis* were found in only one sample.[17] However, the species *C. xiaoi* was found both in goats with diarrhea and asymptomatic individuals. Therefore, we cannot say which species would be found in our study only through morphometry analysis, as molecular analysis would be required for identification.

All animals parasitized by *Cryptosporidium* spp. in this study had access to pasture and remained in facilities with concrete floors at night. However, the parasitized animals showed a low number of oocysts in the fecal smears, probably because some residues of feces may remain on the concrete floor even in constantly clean facilities. It may have enabled greater contact of the animals with the contaminated environment and facilitated the transmission of the parasite from one animal to another by direct contact (fecal-oral) with fecal residues containing oocysts of *Cryptosporidium* spp., which is one of the main forms of contamination.[33]

The low occurrence of contaminated animals in the environment with a concrete floor and the absence of a suspended floor are related to the presence of hygienic-sanitary management in the sector. Prophylactic and hygiene measures must be taken to prevent the transmission of the parasite, such as the periodic cleaning of facilities with the removal of feces, which is the main source of infection of the disease, and isolation of animals with symptoms of the infection.[34]

The goats evaluated in this study were non-pregnant when the samples were collected, that is, the animals showed no drop in immunity, which is commonly observed during pregnancy or peripartum. Another study showed the presence of the protozoan during the peripartum period in goats and a high occurrence of the parasite in newborn goats.[35] The physical conditions represented by weight and BCS indicated that the parasitized goats in this study had a small variation compared to those non-parasitized, showing that adult animals can be carriers of the infection, without necessarily presenting clinical signs of the disease, such

as apathy or physical weakness. Results related to these variables using adult animals are scarce, as most of the studies are conducted with young animals less than one year old.

5. Conclusions

This is the first record of infection by *Cryptosporidium* spp. in goats in the municipality of Teresina, State of Piauí, Brazil. The occurrence of this parasite was considered low, but sanitary management measures are required to prevent the infection of other animals and avoid production-related losses. Furthermore, there is a need to carry out molecular analysis, such as PCR and sequencing, to identify the species of *Cryptosporidium* that affects goats in that region.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: M. R. A. Oliveira, K. R. Santos and S. C. Sousa Júnior. *Data curation:* M. R. A. Oliveira, C. S. M. Luz, S. C. Sousa Júnior and K. D. S. Bresciani. *Formal analysis:* M. R. A. Oliveira, A. F. Evangelista and G. C. Castro. *Investigation:* M. R. A. Oliveira and C. S. M. Luz. *Methodology:* M. R. A. Oliveira, K. D. S. Bresciani, K. R. Santos and S. C. Sousa Júnior. *Supervision:* M. R. A. Oliveira and S. C. Sousa Júnior. *Writing (original draft):* M. R. A. Oliveira, C. S. M. Luz, A. F. Evangelista, K. R. Santos and G. C. Castro. *Writing (proofreading and editing):* M. R. A. Oliveira, A. F. Evangelista, K. D. S. Bresciani and G. C. Castro.

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