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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

Evaluation of the occurrence of Cryptosporidiosis and giardiasis in domestic cats (*Felis*
Cattus Linnaeus, 1758) from shelters in the state of Sergipe, Brazil

Evaluación de la ocurrencia de Criptosporidiosis y giardiasis en gatos domésticos
(*Felis Cattus* Linnaeus, 1758) de refugios del estado de Sergipe, Brasil

Avaliação da ocorrência de Criptosporidiose e giardíase em gatos domésticos (*Felis*
Cattus Linnaeus, 1758) de abrigos do estado de Sergipe, Brasil

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Running Title: Occurrence of cryptosporidiosis and giardiasis in domestic cats

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ABSTRACT

Domestic felines are prevalent in areas of human occupation, even when they are not kept as pets. In view of the above, the present study aims to evaluate the occurrence of cryptosporidiosis and giardiasis in domestic cats from shelters in the state of Sergipe, Brazil. For this purpose, fecal parasitological analyses were performed on cats from shelters, colonies, and NGOs using the Willis-Mollay and centrifugation-sedimentation techniques with Ziehl-Neelsen staining for the identification of gastrointestinal parasites. Of the samples collected, 60,87% were positive for one or more parasites. *Cryptosporidium* sp. had a prevalence of 23,9% in the feline population studied, followed by *Giardia duodenalis* with 4,3%. Other parasitic agents were identified, namely *Ancylostoma* sp., *Toxocara* sp., and *Cystoisospora* sp. The results demonstrate the circulation of protozoa with zoonotic potential, as well as co-infecting parasites, in feline populations from shelters, colonies, and NGOs in the state of Sergipe.

Keywords: Cats – Felines – Parasites – Zoonoses

Resumo

Felinos domésticos são prevalentes em áreas de ocupação humana, mesmo quando não são mantidos como animais de estimação. Diante do exposto, o presente trabalho tem como objetivo avaliar a ocorrência de criptosporidiose e giardíase em gatos domésticos de abrigos do estado de Sergipe, Brasil. Para isso, foram realizadas análises parasitológicas fecais de gatos provenientes de abrigos, colônias e ONGs através das técnicas de Willis-Mollay e de centrifugo-sedimentação com a coloração de Ziehl-Neelsen para a identificação de parasitos gastrointestinais. Das amostras coletadas, 60,87% foram positivas para um ou mais parasitos. *Cryptosporidium* sp. apresentou prevalência de 23,9% na população de felinos estudada, seguida de *Giardia duodenalis* com 4,3%. Outros agentes parasitários foram identificados, sendo *Ancylostoma* sp., *Toxocara* sp. e *Cystoisospora* sp. Os resultados demonstram a circulação de protozoários com potencial zoonótico, bem como parasitos coinfectantes em populações de felinos em abrigos, colônias e ONG's no estado de Sergipe.

Palavras-chave: Felinos – Gatos – Parasitos – Zoonoses

Resumen

Los felinos domésticos son prevalentes en áreas de ocupación humana, incluso cuando no se mantienen como animales de compañía. Ante lo expuesto, el presente trabajo tiene como objetivo evaluar la ocurrencia de criptosporidiosis y giardiasis en gatos domésticos de refugios del estado de Sergipe, Brasil. Para ello, se realizaron análisis parasitológicos fecales de gatos provenientes de refugios, colonias y ONG mediante las técnicas de Willis-Mollay y centrifugación-sedimentación con tinción de Ziehl-Neelsen para la identificación de parásitos gastrointestinales. De las muestras recolectadas, el 60,87% fueron positivas para uno o más parásitos. *Cryptosporidium* sp. presentó una prevalencia del 23,9% en la población de felinos estudiada, seguida de *Giardia duodenalis*. con un 4,3%. Se identificaron otros agentes parasitarios, siendo *Ancylostoma* sp., *Toxocara* sp. y *Cystoisospora* sp. Los resultados demuestran la circulación de protozoos con potencial zoonótico, así como de parásitos coinfectantes, en poblaciones de felinos de refugios, colonias y ONG en el estado de Sergipe.

Palabras clave: Felinos – Gatos – Parásitos – Zoonosis

INTRODUCTION

Domestic cats (*Felis catus* Linnaeus, 1758) are highly prevalent in areas of human occupation, even when they are not kept as pets. They frequently inhabit parks, farms, parking lots, and universities, where they find shelter and food sources (Vitale, 2022; Luzardo *et al.*, 2023). Stray cat populations can reproduce rapidly, making it necessary to adopt population control measures in order to prevent the spread of zoonoses such as toxoplasmosis, giardiasis, sporotrichosis, and rabies (Ferreira *et al.*, 2024).

Parasitic diseases, considered the most neglected zoonotic problems in cats, mainly involve two genera of protozoa, *Giardia* and *Cryptosporidium* (Procesi *et al.*, 2023). Giardiasis and cryptosporidiosis are common protozoal enteric infections in felines and are frequently discussed together (Coelho *et al.*, 2023). They share similar transmission routes (fecal-oral, through the dissemination of cysts or oocysts, eliminated in feces) and overlapping clinical signs, differing significantly in terms of zoonotic potential (Blasco *et al.*, 2021; Barbosa *et al.*, 2023).

Giardiasis in cats is caused by the parasite *G. duodenalis* (Lambl, 1859), a flagellated protozoan that colonizes the small intestine. Hosts are generally infected by ingesting cysts present in contaminated water or food, or through grooming behavior after contact with infected feces (Guadano *et al.*, 2022). Infected hosts may be asymptomatic carriers or may present clinical signs ranging from chronic or intermittent diarrhea, frequently pale, soft, and mucoid, to cachexia, especially in kittens or immunosuppressed patients (Sun *et al.*, 2023).

Feline cryptosporidiosis is typically caused by the protozoan *F. cattus*, an apicomplexan parasite that infects the brush border of intestinal epithelial cells, not living freely in the lumen like *Giardia* spp. (Karimi *et al.*, 2023). Infection occurs through the ingestion of oocysts, which are extremely resistant to chemical disinfectants and environmental degradation (Koseoglu *et al.*, 2022). Most infections in felines are subclinical, with clinical alterations characterized by watery diarrhea, more pronounced in young hosts, immunocompromised hosts, or those with concomitant FIV/FelV infection (Mukbel *et al.*, 2024).

In view of the above, the present study aims to evaluate the occurrence of cryptosporidiosis and giardiasis in domestic cats (*F. cattus*) from shelters in the state of Sergipe, Brazil.

MATERIALS AND METHODS

Study Area

The study was conducted with felines from shelters, colonies and/or Non-Governmental Organizations (NGOs) in the municipalities of Aracaju, São Cristóvão, Nossa Senhora da Glória, and Canindé do São Francisco in the state of Sergipe (10°13'06" S/37°25'13" W), Brazil (Figure 1).

Research Design

To achieve the proposed objectives, fecal samples from felines of different breeds, sexes, and age groups over 1 month of age were used. All animals were evaluated according to the following steps: 1) Anamnesis; 2) Physical examination; 3) Collection of biological samples (feces) for diagnostic purposes of gastrointestinal parasitoses. When the number of animals per enclosure was greater than five animals/pen, 01 pool of fecal samples was collected (for every 5 animals).

All clinical changes observed in the animals were characterized and described in individual records. It is worth noting that the sampling used was non-probabilistic convenience sampling, obtaining the largest possible number of fecal samples from the felines present on the property.

Sample Collection

A total of 46 fecal samples from cats were collected, with the material being placed in an identified collection container and subsequently stored in insulated boxes at 4°C and then sent for processing at the Parasitology Laboratory of the Veterinary Hospital of the Federal University of Sergipe (UFS), Brazil.

Parasitological Evaluation

For the diagnosis and identification of gastrointestinal parasites, two different fecal coproparasitological techniques were used, namely the Willis-Mollay technique and

the centrifugation-sedimentation technique with Ziehl-Neelsen staining for the identification of *Cryptosporidium* sp.

a) Willis-Mollay Technique

The Willis-Mollay method is based on the principle of flotation of helminth eggs and cysts/oocysts of protozoa considered light, involving a hypersaturated sucrose or saline solution (Oliveira *et al.*, 2017).

Five g of the fecal sample were weighed and added to 20 mL of sucrose solution in a tube, followed by the homogenization process. Subsequently, the obtained solution was subjected to sieving, with the objective of filtering the sample and removing larger solid residues. The filtered material was transferred to another test tube, filling the volume until the formation of a convex meniscus. A slide was positioned over the tube so that it came into direct contact with the meniscus. The sample remained at rest for approximately 15 minutes, allowing the parasite eggs to rise and adhere to the slide. After this period, the slide was removed from the tube, and a coverslip was placed over it for microscopic analysis using the 100X and 400X objectives.

b) Modified Centrifugation-Sedimentation Technique with Ziehl-Neelsen Staining

Through this technique, greater identification of *Cryptosporidium* sp. oocysts can be achieved, which, due to their small size, present difficulties in detection (Ritchie, 1948).

Five g of feces were added to 20 mL of distilled water, followed by homogenization and then the sieving process. The filtered material was transferred to a test tube and subjected to centrifugation for 5 minutes at 10,000 rpm. After this procedure, the supernatant was discarded, leaving only the sediment in the tube, which constituted the sample intended for analysis. With the aid of a pipette, part of the sediment was deposited onto the slide, which was placed to dry by heat. After drying, the smear was stained, beginning with the addition of 0.5% fuchsin, which remained in

contact with the sample for 5 minutes. Subsequently, the slides were washed under running water and covered with acid-alcohol for 1 minute, and then washed again with water. Subsequently, the slide was covered with 0.5% methylene blue for 3 minutes, followed by another wash under running water. After final drying, immersion oil was added to the slides for microscopic analysis using the 1000X objective.

Statistical Analyses

The data obtained were recorded in Microsoft Excel 2 spreadsheets and statistically evaluated using R© software, the X2 test, binomial test, Kendall's Tau-b test, Kendall's Tau-c test, and relative and absolute frequency.

Ethical Aspects: The present study was approved by the Ethics Committee on the Use of Animals of the Federal University of Sergipe (No. 3123080523). The participation of the animals was carried out through contact with the shelter, which agreed to participate voluntarily, upon signing an Informed Consent Form, following the guidelines outlined in Resolution No. 466/2012 of the National Commission for Ethics in Research of the National Health Council of the Ministry of Health.

RESULTS

Regarding the prevalence of samples collected by sex, 23,9% (n=11/46) were females, followed by males (17,4%; n=8/46) and both sexes in collective boxes (58,7%; n=27/46) (Table 1).

Regarding the prevalence of clinical alterations in felines, 28,3% (n=13/46) presented diarrhea, followed by rhinotracheitis (4,3%; n=2/46), skin scaling (4,3%; n=2/46), nasal ulcer (4,3%; n=2/46), perianal scaling lesion (4,3%; n=2/46), conjunctivitis (2,2%; n=1/46), ocular corneal ulcer (2,2%; n=1/46), lacerative dermatological lesions (2,2%; n=1/46) and alopecia (2,2%; n=1/46). 71,7% (n=33/46) of the individuals did not present clinical alterations.

Regarding the prevalence of feces consistency per animal, 65,2% (n=30/46) presented feces of normal consistency, followed by soft (28,3%; n=13/46) and dry (6,5%; n=3/46). Regarding the prevalence of animals positive for the presence of gastrointestinal parasites, 60,87% (n=28/46) were positive for the presence of gastrointestinal parasites. Regarding the prevalence of gastrointestinal parasites and co-infections diagnosed in fecal samples, 23,9% (n=11/46) were *Cryptosporidium* sp., followed by *Ancylostoma* sp. (15,2%; n=7/46), *Cryptosporidium* sp. and *Ancylostoma* sp. (8,7%; n=4/46), *G. duodenalis* (4,3%; n=2/46), *Ancylostoma* sp., *Toxocara* sp. and *Cryptosporidium* sp. (4,3%; n=2/46), *Toxocara* sp. and *Cryptosporidium* sp. (2,2%; n=1/46), and *Cystoisospora* sp., *Ancylostoma* sp., *Toxocara* sp. and *Cryptosporidium* sp. (2,2%; n=1/46) (Figure 2 and Table 2).

DISCUSSION

The present work evaluates the occurrence of cryptosporidiosis and giardiasis in domestic cats (*F. cattus*) located in shelters and colonies of municipalities in the state of Sergipe, Brazil.

The presence of sick felines together with animals without the presence of clinical alterations is notable, in addition to individuals positive for the presence of gastrointestinal parasites (60,87%; n=28/46). Parasitized hosts represent an important source of maintenance of the cycle of cryptosporidiosis, giardiasis and the co-parasitic agents detected (Gavioli *et al.*, 2011). These release cysts and eggs in their feces, contaminating the environment and making domestic felines that share the same environment in shelters susceptible (Tandel *et al.*, 2019; Berenguer *et al.*, 2021; Paula *et al.*, 2021).

Compared to the study carried out by Mosallanejad (2010), detecting *G. duodenalis* in 2% of the feline population, the present work identified a significant

prevalence of the protozoan (4,3%) in cat populations. The detection of *G. duodenalis* cysts in the present study proves the maintenance of the parasite transmission cycle in shelters located in urban centers of municipalities in Sergipe, these characterized by the maintenance of cats in situations of vulnerability. It is worth mentioning that animals confined in facilities with precarious sanitary conditions are susceptible to reinfection during self-grooming due to the presence of cysts in their fur, in addition to the presence of feces contaminated by cysts of the parasite (Anjos *et al.*, 2013; Procesi *et al.*, 2023; Veyna-Salazar *et al.*, 2023).

Worldwide, the prevalence of *Cryptosporidium* sp. in domestic canines and felines is considered to be around 10% (Barbosa *et al.*, 2023). In the present study, it was possible to observe an isolated prevalence above the global reference value, being detected in 23,9% of the felines from Sergipe, in addition to its association with other gastrointestinal parasitic agents. This perspective serves as a warning for the dissemination of cryptosporidiosis among animals and human beings in the municipalities of Sergipe, since the maintenance of the cycle between domestic animals and responsible guardianship has already been detected in previous studies, becoming a risk of contamination for professionals who perform activities in the shelters (Taghipour *et al.*, 2021; Coelho *et al.*, 2023).

As in the present work, research previously carried out in states of the Southeast and Midwest detected the presence of parasites such as *Ancylostoma* sp. and *Cystoisospora* sp. in fecal samples from felines, using flotation techniques in sucrose and ZnSO₄ (Gavioli *et al.*, 2011; Ramos *et al.*, 2020; Silva *et al.*, 2023). Furthermore, in the present work, the presence of *Toxocara* sp. together with *Ancylostoma* sp. and *Cystoisospora* sp. in association with *Cryptosporidium* sp. was detected. The presence of these agents in an isolated form and in association represents a risk to environmental, human, and animal health (Berenguer *et al.*, 2021). These have a worldwide distribution, contaminate mammals through the ingestion of eggs, cysts, and oocysts present in the

soil and in food, triggering clinical manifestations characterized by gastroenteritis in their hosts, as observed in 28,3% of the felines studied (Paula *et al.*, 2021; Bonilla-Aldana *et al.*, 2024).

The results of the study demonstrate the circulation of protozoa with zoonotic potential and associated parasites in cat populations. Such findings point to the need for effective prevention and control measures, including regular deworming protocols and improvements in the sanitary management of felines in situations of vulnerability in the state of Sergipe.

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285

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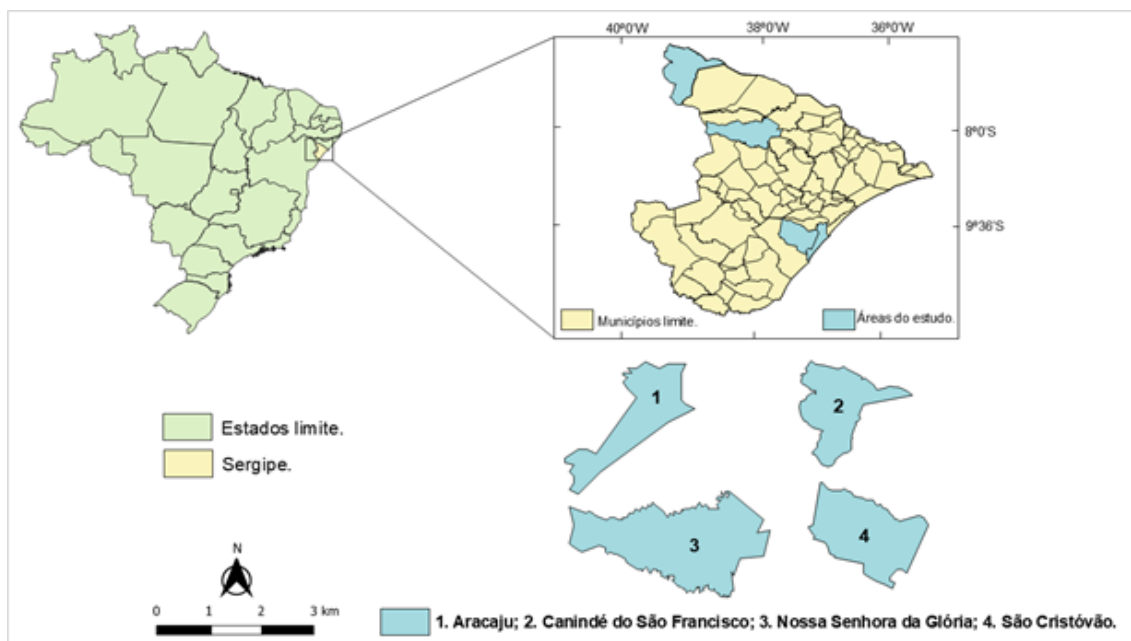


Figure 1. Map of Brazil highlighting the state of Sergipe, where the research was carried out, followed by the municipalities of Aracaju (1), Canindé do São Francisco (2), Nossa Senhora da Glória (3), and São Cristóvão (4).

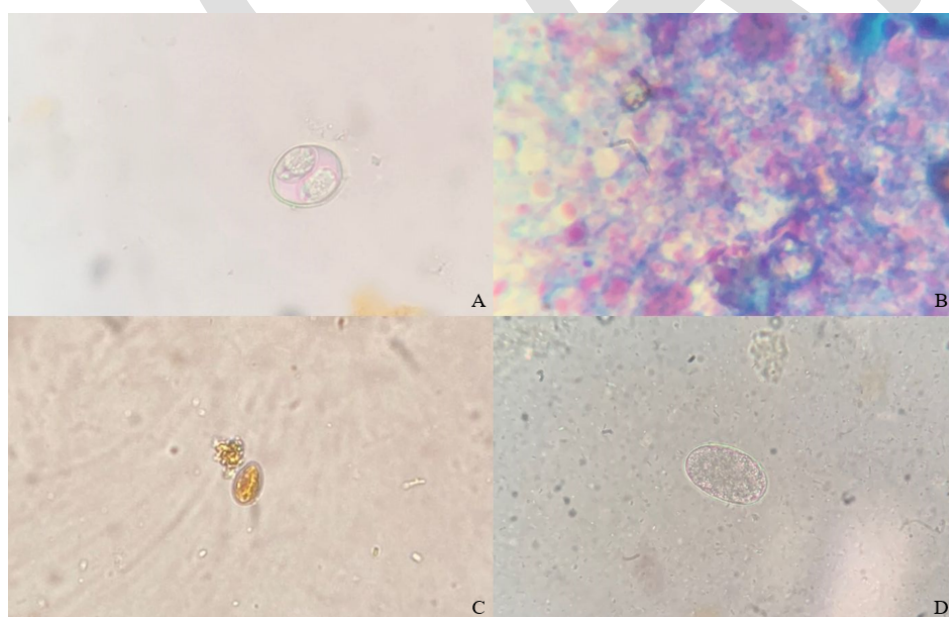


Figure 2. Identified parasites. A. Oocyst of *Cystoisospora* sp., B. Oocysts of *Cryptosporidium* sp., C. Oocyst of *Giardia* sp., D. Egg of *Ancylostoma* sp. Source: (Personal Archive 2024/2025).

377 **Table 1.** Prevalence of samples collected according to the age group of animals from
 378 shelters, NGOs, and colonies.

Age group	AF	RF (%)
Kitten	8	17.4
Young	5	10.9
Adult	13	28.3
Young and Adult	3	6.5
Kitten, Young and Adult	17	37
Total	46	100%

379

380 **Table 2.** Detection of parasites related to the sex parameter and their statistical results.

Sex	Parasites	Positive	Negative	X ²	Tau-b Kendall	Fi
Female	<i>G. duodenalis</i>	*	6	0.44	0,12	0.44
Male		*	2			
Female and Male		2	9			
Female	<i>Ancylostoma</i> sp.	1	6	0.44	0.56	0.44
Male		2	2			
Female and Male		4	9			
Female	<i>Cryptosporidium</i> sp.	1	6	0.25	0.25	0.06
Male		1	2			
Female and Male		9	9			

381