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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

EPIDEMIOLOGICAL ASPECTS OF BRONCHOPULMONARY AND GASTROINTESTINAL PARASITE INFECTION IN CATS FROM NORTHEAST BRAZIL

ASPECTOS EPIDEMIOLÓGICOS DE LA INFECCIÓN POR PARÁSITOS BRONCOPULMONARES Y GASTROINTESTINALES EN GATOS DEL NORDESTE DE BRASIL

ASPECTOS EPIDEMIOLÓGICOS DA INFECÇÃO POR PARASITAS BRONCOPULMONARES E GASTROINTESTINAIS EM GATOS DO NORDESTE DO BRASIL

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Running Head: Apidemiological aspects of parasite infection in cats

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ABSTRACT

This study investigated the presence of bronchopulmonary and gastrointestinal parasites in cats from the state of Sergipe, Brazil, highlighting their medical and veterinary importance. Fecal samples from 100 felines were analysed using coproparasitological techniques, showing a 62% positivity rate for parasites, with a significant prevalence of gastrointestinal species. Notably, bronchopulmonary parasites, particularly *Aelurostrongylus* sp., were detected for the first time in Sergipe. Samples were analysed through the Mini-FLOTAC® and Baermann techniques, which proved effective in

detecting various parasite types, including eggs, larvae, and cysts. Clinical evaluations were performed, with clinical signs observed in less than 25% of infected cats. The study identified eight parasite genera, with *Ancylostoma* sp. and *Strongyloides* sp. among the most prevalent gastrointestinal parasites, while *Aelurostrongylus* sp. represented bronchopulmonary infections. Both monoparasitism and polyparasitism were recorded, with Mini-FLOTAC® showing superior diagnostic performance. This research presents evidence of *Aelurostrongylus* sp. in domestic cats in Sergipe and underscores the need for enhanced veterinary surveillance. Findings provide a foundation for understanding the transmission dynamics and parasite-host relationships of these diseases, contributing significantly to the epidemiological knowledge in the region.

Keywords: Bronchopulmonary parasites - clinical alterations - feline parasites - Mini-FLOTAC®

RESUMEN

Este estudio investigó la presencia de parásitos broncopulmonares y gastrointestinales en gatos del estado de Sergipe, Brasil, destacando su importancia médica y veterinaria. Se analizaron muestras fecales de 100 felinos mediante técnicas coproparasitológicas, revelando una tasa de positividad del 62% para parásitos, con una prevalencia significativa de especies gastrointestinales. De forma relevante, los parásitos broncopulmonares, particularmente *Aelurostrongylus* sp., fueron detectados por primera vez en Sergipe. Las muestras se analizaron mediante las técnicas Mini-FLOTAC® y Baermann, que demostraron ser eficaces en la detección de diversos tipos de parásitos, incluidos huevos, larvas y quistes. Se realizaron evaluaciones clínicas, observándose signos clínicos en menos del 25% de los gatos infectados. El estudio identificó ocho géneros de parásitos, siendo *Ancylostoma* sp. y *Strongyloides* sp. los parásitos gastrointestinales más prevalentes, mientras que *Aelurostrongylus* sp. representó las

infecciones broncopulmonares. Se registraron casos de monoparasitismo y poliparasitismo, presentando la técnica Mini-FLOTAC® un rendimiento diagnóstico superior. Esta investigación aporta evidencias de la presencia de *Aelurostrongylus* sp. en gatos domésticos de Sergipe y refuerza la necesidad de una vigilancia veterinaria más rigurosa. Los resultados proporcionan una base para la comprensión de la dinámica de transmisión y de las relaciones parásito-hospedador de estas enfermedades, contribuyendo de manera significativa al conocimiento epidemiológico de la región.

Palabras clave: Alteraciones clínicas - parásitos broncopulmonares - parásitos de felinos - Mini-FLOTAC®

RESUMO

Este estudo investigou a presença de parasitas broncopulmonares e gastrointestinais em gatos do estado de Sergipe, Brasil, destacando a sua importância médica e veterinária. Amostras fecais de 100 felinos foram analisadas através de técnicas coproparasitológicas, revelando uma taxa de positividade de 62% para parasitas, com prevalência significativa de espécies gastrointestinais. De forma relevante, parasitas broncopulmonares, particularmente *Aelurostrongylus* sp., foram detectados pela primeira vez em Sergipe. As amostras foram analisadas pelas técnicas Mini-FLOTAC® e Baermann, que se mostraram eficazes na deteção de diversos tipos de parasitas, incluindo ovos, larvas e quistos. Foram realizadas avaliações clínicas, tendo sido observados sinais clínicos em menos de 25% dos gatos infetados. O estudo identificou oito géneros de parasitas, sendo *Ancylostoma* sp. e *Strongyloides* sp. os parasitas gastrointestinais mais prevalentes, enquanto *Aelurostrongylus* sp. representou as infeções broncopulmonares. Registaram-se casos de monoparasitismo e poliparasitismo, com a técnica Mini-FLOTAC® a apresentar desempenho diagnóstico superior. Esta investigação apresenta evidências da presença de *Aelurostrongylus* sp.

em gatos domésticos em Sergipe e reforça a necessidade de uma vigilância veterinária mais rigorosa. Os resultados fornecem uma base para a compreensão da dinâmica de transmissão e das relações parasita-hospedeiro destas doenças, contribuindo de forma significativa para o conhecimento epidemiológico na região.

Palavras-chave: Alterações clínicas - parasitos broncopulmonares - parasitos de felinos - Mini-FLOTAC®

INTRODUCTION

In feline medicine, parasitic diseases are increasingly prevalent and considered significant causes of clinical manifestations. Cats can be affected by various gastrointestinal helminths, such as *Ancylostoma* sp., *Toxocara cati* (Schrank, 1788), and *Dipylidium caninum* (Linnaeus, 1758). However, species of nematodes *Ancylostoma* sp. and *Toxocara cati* (Schrank, 1788) are more prevalent (Abbas *et al*., 2022; Filho *et al*., 2022; Silva *et al*., 2023). The diagnosis and study of emerging or neglected parasitic infections have been one of the major challenges in small animal medicine (Abbas *et a*l., 2022).

Bronchopulmonary parasites, for example, have sparked great interest among veterinarians and parasitologists worldwide due to the increase in the number of cases of felines infected with bronchopulmonary nematodes in several European countries and the occurrence of the parasitosis in regions that were previously non-endemic (Traversa & Guglielmini, 2008; Ferraz *et al*., 2019).

The reasons for the emergence of new cases of bronchopulmonary parasitosis in felines are still unknown; however, some factors such as climate change, intense movement of companion animals, diversity, and susceptibility of potential intermediate hosts (vectors) have been taken into consideration (Livia *et al*., 2023; Yildirim *et al*., 2023).

Pulmonary metastrongylids are among the main agents involved in the infection of companion animals, where *Aelurostrongylus abstrusus* (Railliet, 1898) (Nematoda, Metastrongyloidea, Angiostrongylidae), *Troglostrongylus brevior* (Gerichter, 1948), and *Troglostrongylus subcrenatus* (Railliet, 1913) (Nematoda, Strongylida, Crenosomatidae) are listed as the main pulmonary parasites of domestic felines (Livia *et al*., 2023; Yildirim et al., 2023). The adult forms of these nematodes reside in nodules in the bronchioles and alveolar ducts (Nonnis *et* al., 2023; Napoli *et al*., 2023).

As clinical signs in felines infected with bronchopulmonary parasites are not always detectable, as they may present the disease in its asymptomatic or subclinical form. Parasitological examinations, such as the Baermann and Mini-FLOTAC® methods, should be used for confirmation of the diagnosis (Ianniello *et al*., 2020; Cardoso *et al*., 2021; Marchiori *et al*., 2024). However, it's worth noting that young, debilitated, and/or immunosuppressed animals may present with coughing, sneezing, mucopurulent nasal discharge, dyspnea, tachypnea, exercise intolerance, anorexia, weight loss, and even death (Nonnis *et al*., 2023). When present, clinical signs result from the intense inflammatory response caused by the elimination of eggs by adult females and the migration of first-stage larvae (L1) to the bronchial tree, causing lesions in the pulmonary alveoli, bronchioles, and local arteries (Vismarra *et al*., 2023; Yildirim *et al*., 2023).

Given the impacts that pulmonary and gastrointestinal parasites can have on animal health, the zoonotic potential of some of them, their geographic expansion, and recent advances in epidemiology, clinical, and control of these agents (Abbas *et a*l., 2022), the present study aims to describe the clinical and epidemiological aspects of bronchopulmonary and gastrointestinal parasite infection in naturally infected felines in the state of Sergipe, Brazil.

MATERIALS AND METHODS

Study area

The study included felines residing in rural and/or urban areas of the municipalities of Aracaju, Carira, Nossa Senhora Aparecida, Nossa Senhora das Dores, Nossa Senhora da Glória, and Itabaiana, located in the state of Sergipe, Brazil (10°13'06" S/37°25'13" W) (Figure 1). Sergipe is situated in the Northeast Region and is bordered by the Atlantic Ocean to the east and the states of Bahia to the west and south, and Alagoas to the north, separated by the São Francisco River (IBGE, 2015).

Experimental design and animals

To achieve the proposed objectives, fecal samples were collected from 100 domestic felines of different breeds, sexes, and ages over 2 months old, residing in households, shelters, or Non-Governmental Organizations (NGOs). All animals were clinically evaluated, following three basic steps: 1) Anamnesis and clinical history; 2) Clinical examination; and 3) Fecal sample collection for diagnostic purposes. It is worth noting that all clinical alterations of the animals were described in individual records.

Coproparasitological evaluation

Fecal samples were collected from all felines through spontaneous defecation, with the biological material deposited in properly labeled collection tubes and stored in a cool box at 4°C until further processing at the Clinical Analysis Laboratory of the Universidade Federal de Sergipe - Sertão campus, Brazil. All samples were analyzed using the Mini-FLOTAC® technique (Cringoli *et al*., 2013) and the Baermann "gold standard" method (Willesen, 2021).

The Mini-FLOTAC® technique was performed by weighing two grams of feces, which were then deposited into the Fill-FLOTAC® containing 18 mL of zinc sulfate flotation solution ($ZnCl_2$ at 1.350; S7). Subsequently, the process of homogenization and sieving was carried out, and then 5 mL of the solution was transferred to the chamber of the Mini-FLOTAC® until a positive meniscus formed at the end of each chamber. After 10 minutes, the central disk of the Mini-FLOTAC® was rotated at a 90° angle with the

aid of a key located at the end of the plate, which was then removed so that the chamber could be attached to the optical microscope adapter. Identification and counting of parasitic structures were then carried out at magnifications of 100X and 400X (Lima *et al*., 2015).

The Baermann technique was performed by weighing approximately 10g of feces, which were then wrapped in gauze to form a pouch, attached to a rod, and then deposited over a sedimentation glass containing water, ensuring that the fecal sample was fully submerged. After an overnight process, 1.5 mL of sediment from the bottom of the glass was pipetted and deposited into a Falcon tube, which was then centrifuged at a speed of 1500-2000 rpm for 3 min. Following centrifugation, the supernatant was discarded, and approximately 0.1 mL of sediment from the tube was pipetted onto a slide and cover slip (in triplicate), then examined under an optical microscope at magnifications of 100X and 400X. If necessary, one or two drops of Lugol's solution were added to fix the larvae (Alho *et al*., 2013).

All parasites observed in the fecal samples were photographed and analyzed using ImageJ software to obtain morphological and morphometric characteristics, and were then identified based on the taxonomic keys provided by Bowman *et al*. (2006) and Taylor *et al*. (2010).

Statistical analysis

All data were analyzed using InStat software (GraphPad Software), calculating absolute frequency (n/N) and relative frequency (%). The Chi-square test (X^2) was used to determine if there was significance between the diagnostic methods and clinical alterations observed in the animals. The independent variables considered in the model will be those with statistical significance less than 0.20. EpiInfoTM7 will also be used to perform statistical calculations, with a significance level established at $p < 0.05$.

208 ***Ethical aspects***

209 The present research was submitted to and approved by the Ethics Committee
210 on Animal Use of the Universidade Federal de Sergipe (approval number 4813080621).
211 Animal participation in the research was voluntary, with felines becoming participants
212 after the responsible guardian signed the Informed Consent Form, following the
213 guidelines outlined in Resolution No. 466/2012 of the National Commission for Ethics in
214 Research of the National Health Council of the Ministry of Health.

215

216 **RESULTS**

217 In this study, parasitological evaluation revealed positivity for gastrointestinal
218 parasites (93.65%), bronchopulmonary parasites (4.76%), and pseudoparasites (1.59%)
219 in 62.00% (n=62/100; $p < 0.0001$) of fecal samples from felines in the municipalities of
220 Aracaju (29.03%; 18/62), Nossa Senhora Aparecida (1.61%; 1/62), Nossa Senhora das
221 Dores (3.23%; 2/62), and Nossa Senhora da Glória (66.13%; n=41/62) ($p < 0.0008$).

222 All biological samples from cats in the municipalities of Carira (0.00%; 0/5) and
223 Itabaiana (0.00%; 0/3) were considered negative. The Venn diagram (Figure 2)
224 schematically demonstrates the presence of bronchopulmonary and gastrointestinal
225 parasites in 16.67% and 66.67% ($p < 0.0001$) of felines from the Sergipe municipalities,
226 respectively.

227 Parasitic structures were detected in the forms of eggs (59.30%), oocysts
228 (18.60%), cysts (4.65%), larvae (16.28%), and proglottids (1.16%) ($p < 0.0003$) (Figure
229 12). Eight genera of parasites were identified in the fecal samples of felines (Table 1),
230 with five types of helminths (62.50%) and three protozoa (37.50%) ($p < 0.0026$).
231 Highlighted among the gastrointestinal parasites were *Ancylostoma* sp. (44.00%; 44/62;
232 $p < 0.0011$), *Dipylidium* sp. (1.00%; 1/62; $p < 0.0005$), *Cystoisospora* sp. (19.00%; 19/62;
233 $p < 0.0019$), *Entamoeba* sp. (3.00%; 3/62; $p < 0.0001$), *Giardia* sp. (3.00%; 3/62; $p <$

0.0001), *Strongyloides* sp. (25.00%; 25/62; p < 0.0063), and *Toxocara* sp. (2.00%; 2/62; p < 0.0006) as the most frequent, followed by the bronchopulmonary parasite *Aelurostrongylus* sp. (3.00%; 3/62; p < 0.0001).

Monoparasitism (33.87%; 21/62) was observed exclusively in felines infected only with gastrointestinal parasite genera, while polyparasitism (66.13%; 41/62) (p < 0.0293) was identified in animals with two or more types of enteroparasites, in which symbiotic interactions: bronchopulmonary parasite + gastrointestinal parasite (4.84%; p < 0.0001) and gastrointestinal parasite + gastrointestinal parasite (61.29%; p < 0.0233) were reported.

Regarding the effectiveness of the parasitological methods employed, 98.41% and 25.19% of the samples were positive in the Mini-FLOTAC® and Baermann techniques, respectively (p < 0.0001). 100% of eggs, larvae, cysts, oocysts, and proglottids were diagnosed using the Mini-FLOTAC® technique, while the Baermann method was able to recover only Strongyloides sp. larvae in 12.50% of the analyzed feline samples (p < 0.0001).

Regarding the profile of felines positive for enteroparasites, it was observed that 72.00% were females and 28.00% were males, adults, without defined breed, with an age range between 3 months and 6 years (p < 0.0153). Clinically, only 3.23% (2/62) of the animals had a history of respiratory and/or gastroenteritis symptoms in the last 30 days; however, in the clinical examination, 24.19% (15/62) of the felines presented noteworthy alterations (p < 0.0004) (Figure 3). Clinical alterations were observed in felines positive for bronchopulmonary and/or gastrointestinal parasites (Figure 4).

DISCUSSION

This study reports for the first time the natural infection of domestic felines by bronchopulmonary parasites in Sergipe, in addition to the description of different gastrointestinal parasites that have an impact on both animal and human health.

The positivity rate (62.00%) for parasitic structures detected in fecal samples of felines from Sergipe was higher than those reported in studies conducted in Australia (8.6%) (Mcglade *et al*., 2003), China (41.39%) (Yang & Liang, 2015), Egypt (52.40%) (Abbas *et al*., 2022), United States (5.1%) (Hoggard *et al*., 2019), France (Bourgoin *et al*., 2022), Greece (50.70%) (Symeonidou *et al*., 2018), Italy (22.00%) (Sauda *et al*., 2019), India (19.00%) (Vincy & Tresamol, 2023), Russia (18.20%) (Kurnosova *et al*., 2023), and Zimbabwe (1.1%) (Pfukenyi *et al*., 2010).

When comparing the overall positivity rate for bronchopulmonary and/or gastrointestinal parasites in patients from our study with published data from epidemiological surveys conducted in Brazil, we observed that studies conducted in Pernambuco using the FLOTAC® technique showed a higher percentage of positivity (65.00% to 100%) (Monteiro *et al*., 2016; Lima *et al*., 2017). However, research using traditional techniques (e.g., sucrose flotation, spontaneous sedimentation, and direct examination) showed lower positivity rates: Rio de Janeiro (49.50%) (Pereira *et al*., 2017), São Paulo (18.10%) (Gennari *et al*., 2016), (26.03%), Goiás (17.00% - 60.00%) (Lima *et al*., 2018), Rio Grande do Sul (24.63%) (Moreira *et al*., 2018), and Santa Catarina (37.80%) (Stalliviere *et al*., 2009).

Clinically, less than 25% of naturally infected felines in Sergipe by bronchopulmonary or gastrointestinal parasites presented clinical alterations. Some authors emphasize that certain parasitized animals may be asymptomatic; however, they may develop cutaneous, gastrointestinal, hepatic, and neurological alterations (Simpson, 1998; Lima *et al*., 2021). Arslan *et al*. (2019) also highlight that cats are commonly affected by toxocariasis, a gastrointestinal parasitic disease caused by larvae of the ascarid nematodes, *Toxocara canis* (Stiles, 1905) and *T. cati*, which can lead to gastroenteritis, peritonitis, eosinophilic ascites due to peritoneal inflammation, abdominal pain, and nausea in infected felines.

In feline medicine, respiratory parasitic infections pose a particular challenge for veterinarians due to the number of pathologies with similar clinical manifestations (Stepanović *et al*., 2020). Respiratory alterations were observed exclusively in felines parasitized by the bronchopulmonary helminth *Aelurostrongylus* sp., which presented nasal discharge, coughing, sneezing, and dyspnea, clinical signs that, according to Traversa *et al*. (2010), should be carefully evaluated and considered in the differential diagnosis of feline cardiopulmonary diseases.

Several coproparasitological techniques from the FLOTAC® group are available for the qualitative and/or quantitative diagnosis of gastrointestinal and pulmonary parasitoses in felines. However, some studies highlight the importance of using the Baermann technique in detecting larvae of bronchopulmonary parasites *Aelurostrongylus abstrusus* and *T. brevior*, which were detected only by the Baermann test (Colombo *et al*., 2022). Despite the Mini-FLOTAC® technique showing the best performance in diagnosing mixed infections (gastrointestinal and pulmonary), the Baermann test is considered the gold standard for diagnosing cardiopulmonary nematode parasites in animals, due to the positive hydro/thermotropism demonstrated by first-stage larvae present in fecal samples (Morelli *et al*., 2022).

The findings of this study highlight the presence of bronchopulmonary parasites in the state of Sergipe, as well as the presence of gastrointestinal parasite genera with medical and veterinary significance. Additional studies are warranted to elucidate the parasite-host relationship and the transmission dynamics of these parasitic diseases in Sergipe.

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Table 1. Percentage of positivity by type of parasite detected in populations of cats in Northern Brazil.

Parasite/Parasitic structure detected	Absolute Frequency (n/N)	Relative Frequency (%)	Prevalence
Aelurostrongylus sp. [L]	3/62	3.00	3
Ancylostoma sp. [E]	44/62	44.00	44
Cystoisospora sp. [O]	19/62	19.00	19
Dipylidium sp. [E,P]	1/62	1.00	1
Entamoeba sp. [C]	3/62	3.00	3
Giardia sp. [C]	3/62	3.00	3
Strongyloides sp [E]	25/62	25.00	25
Toxocara sp. [E]	2/62	2.00	2

Note: E – Eggs, C – Cyst, O – Oocyst, L – Larvae. P – Proglottis

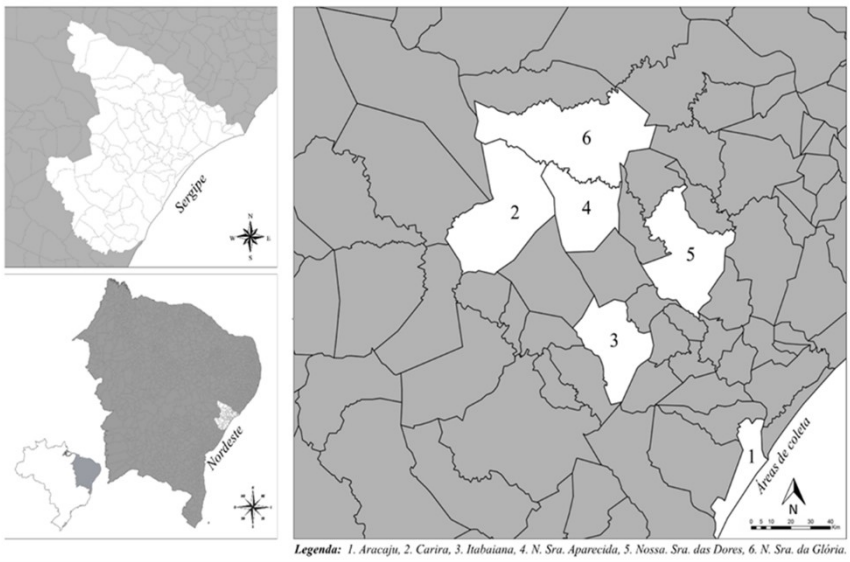
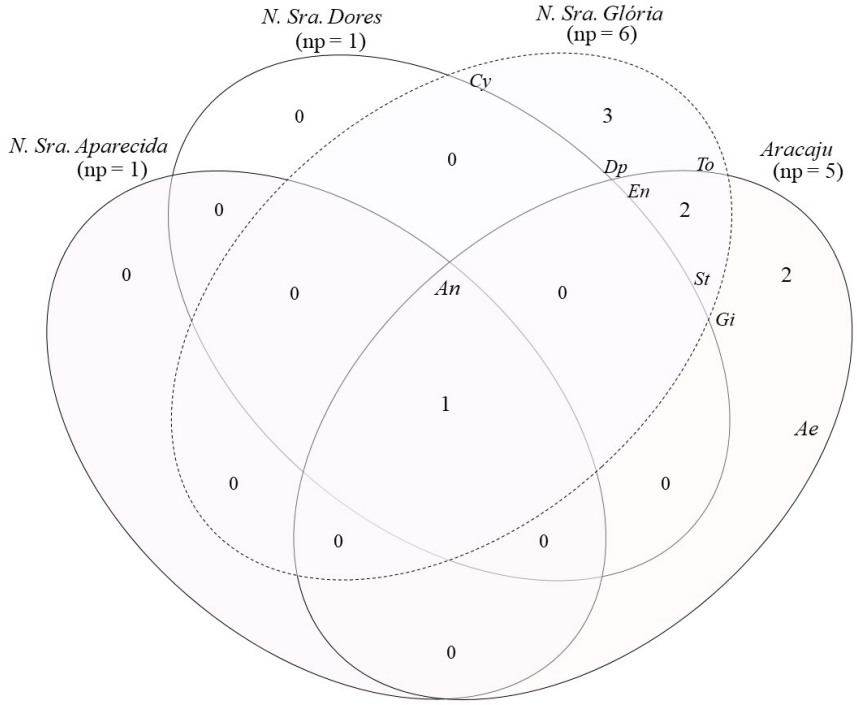


Figure 1. Topographic location of the collection areas in the state of Sergipe, Northern Brazil.



Legenda: np – Número de parasitos; *Ae* – *Aelurostrongylus* sp.; *An* – *Ancylostoma* sp.; *Cy* – *Cystoisospora* sp.; *Dy* *Dipylidium* sp.; *Gi* – *Giardia* sp.; *En* – *Entamoeba* sp.; *St* – *Strongyloides* sp.; *To* – *Toxocara* sp..

Figure 2. Venn diagram schematically illustrating the presence of bronchopulmonary and gastrointestinal parasites in populations of felines from four municipalities in Sergipe, Northern Brazil.

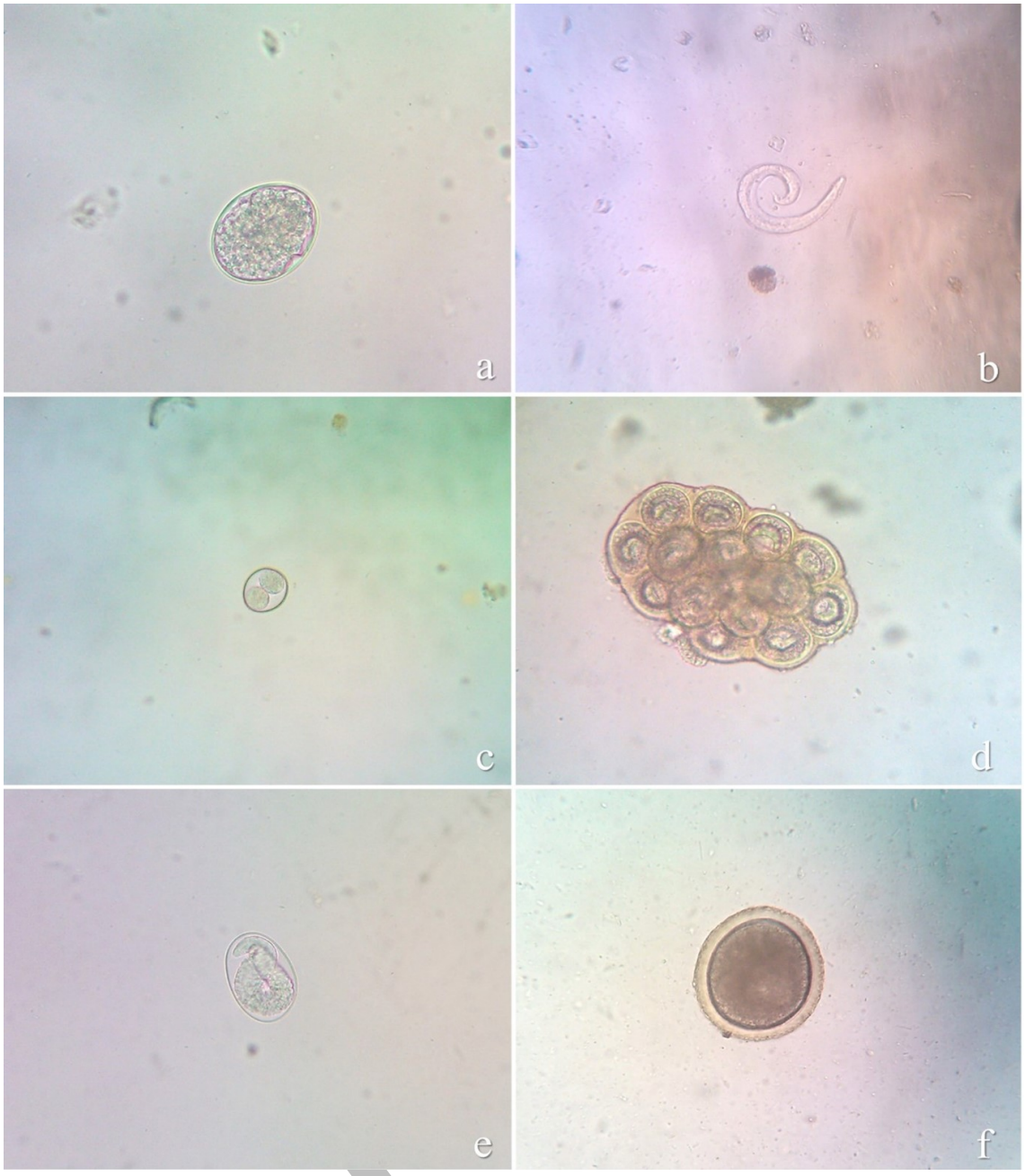


Figure 3. Parasitic bronchopulmonary and gastrointestinal parasites diagnosed in cats. a - *Ancylostoma* sp. egg; b - *Aelurostrongylus* sp. larva; c - *Cystoisospora* sp. oocyst; d - *Dipylidium* sp. egg capsule; e - *Strongyloides* sp. larvated egg; f - *Toxocara* sp. egg.

Figure 4. Clinical alterations observed in felines positive for bronchopulmonary and/or gastrointestinal parasites. a) Animal presenting ocular and nasal discharge; b) Feline presenting cachexia; c) Cat presenting apathy and prostration; and d) Feline presenting weight loss and skin lesions throughout the body.