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ORIGINAL ARTICLE

Prevalence of Cryptosporidiosis in domestic and stray cats in Mymensingh, Bangladesh

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Abstract

Background: Cryptosporidium sp. infection is a significant zoonotic disease affecting both humans and animals, including cats. The primary objectives of this research were to determine the prevalence of Cryptosporidium sp. infection and to identify the pathological lesions caused by the infection in domestic and stray cats in the Mymensingh district, Bangladesh.

Methods: In this study, fecal samples from 36 cats were collected and analyzed using both direct smear and safranin staining methods to detect Cryptosporidium oocysts. Additionally, the intestines of euthanized cats were examined for gross and microscopic lesions. The study was performed at the Department of Parasitology and the Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Results: A total of 36 adult cats (16 males and 20 females) were examined. Among them, three cats (8.3%; 95% Confidence Interval: 2.2–23.6) tested positive for Cryptosporidium sp. infection. The prevalence was higher in young cats under 1 year of age (16.6%) compared to adult cats over 1 year (4.1%). Females showed a higher prevalence (10%) compared to males (6.2%). The infection was detected throughout the year except during the winter season. Prevalence was higher during the summer season (15.4%) compared to the rainy season (8.3%). However, none of the observed differences in prevalence by age, sex, or season were statistically significant.

Conclusion: The study highlights that Cryptosporidium sp. infection is a health concern for both domestic and stray cats, regardless of their age, sex, or season. Regular screening and treatment of domestic cats are recommended to reduce the risk of zoonotic transmission to humans.

Keywords: *Cryptosporidium* sp., age, pathology, season, sex

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Introduction

Cryptosporidium sp. is a zoonotic pathogen of significant importance for the health of cats, other animals, and people. Humans and animals become infected by ingesting the oocysts of the organism. Among the various species, *Cryptosporidium parvum* is the only known one to cause clinical disease in both domestic animals and humans. Unlike other members of the *Eimeriidae* family, *Cryptosporidium* sp. does not invade host cells and lacks strict host specificity, which allows for cross-infection between domestic animals, laboratory animals, and humans (Urquhart *et al*., 1996; Jacobs, 1985). The development of schizonts and gamonts of *Cryptosporidium* sp. occurs within a parasitophorous envelope, apparently derived from the microvilli of the intestinal epithelium. As a result, the cellular dissolution typically observed with other coccidia does not occur with *Cryptosporidium* sp.. Infections lead to mucosal changes in the ileum, including villous stunting, swelling, and fusion. These changes negatively impact the activity of certain membrane-bound enzymes (Soulsby, 1982).

Clinically, the disease is characterized by anorexia and intermittent diarrhea, which can result in poor growth rates. In humans, immune-compromised individuals, especially those with AIDS, are particularly vulnerable to severe cryptosporidiosis, often exacerbated by repeated auto-infections (Soulsby, 1982).

In Bangladesh, domestic and stray cats are widespread, particularly in Mymensingh, where cat ownership is common. This close association between cats and humans raises concerns about the zoonotic transmission of *Cryptosporidium* sp.. Despite this, no prior studies have been conducted on the prevalence of *Cryptosporidium* sp. in cats in Bangladesh. Factors such as the association of infection with age, sex, seasonal variation, and the pathological lesions caused by *Cryptosporidium* sp. in cats remain unexplored in the country.

Therefore, this study aimed to determine the prevalence of *Cryptosporidium* sp. infection in domestic and stray cats in Bangladesh. Additionally, it sought to identify gross and microscopic pathological lesions associated with the infection.

Materials and methods

Sample collection

A total of 36 domestic and stray cats were collected from the Bangladesh Agricultural University and surrounding areas in the Mymensingh district, Bangladesh, from July 2006 to June 2007. The study was based on fecal, autopsy, and histopathological examinations.

Fecal Sample Examination

Fecal samples, weighing 10–15 grams, were collected from the cages after the cats defecated early in the morning. The samples were examined using both the direct smear method and concentration techniques, including centrifugation and saturated salt solution flotation methods, as described by Soulsby (1982). For the direct smear examination, a small amount of fecal material was placed on a clean glass slide, mixed with normal saline, and smeared evenly. The slide was then examined under a microscope.

For the centrifugation method, five grams of feces were mixed with distilled water in a centrifuge tube, and the mixture was sieved into another test tube. This tube was centrifuged at 1500 rpm for three minutes. The sediment or pellet was collected using a dropper, placed on a clean glass slide, and examined under a microscope.

In the saturated salt solution flotation method, five grams of feces were mixed with a saturated sodium chloride solution in a test tube. The mixture was sieved into another test tube, which was then filled with flotation fluid. A cover slip was placed over the opening of the tube and left undisturbed for 10 minutes to allow the oocysts to float to the surface.

The cover slip was then carefully transferred to a clean glass slide for microscopic examination. All fecal samples were analyzed under various magnifications, including 10X, 40X, and 100X.

Purification and staining for Cryptosporidium sp.

For purification, two grams of feces were mixed with eight milliliters of phosphate-buffered saline (PBS, pH 7.2) in a Falcon tube and centrifuged at 500 rpm for 10 minutes. The clear supernatant was discarded, and the

sediment was resuspended in PBS to a final volume of eight milliliters. To this, four milliliters of ether were added, and the mixture was shaken thoroughly. After settling, four distinct layers formed, with the sediment collecting at the bottom. The upper three layers were carefully removed and discarded.

For staining, a fecal smear was prepared on a clean glass slide and air-dried. The slide was quickly passed through a flame to fix the oocysts. The smear was then fixed in 3% HCl in methanol for five minutes and washed with tap water. The slide was flooded with 1% safranine and heated until steaming, after which it was left for three minutes before being washed again. A counterstain of 1% alkaline methylene blue was applied for 30 seconds, followed by a final wash with tap water. The slides were air-dried and examined under a high-power microscope (100X) with oil immersion.

Examination of intestinal lesions

The intestines of euthanized cats were thoroughly examined for gross lesions. Suspected sections of the intestines were collected for histopathological examination and processed following standard protocols described by Luna (1968).

Data analysis

The age (< 1 year, >1 year), sex, and season (Summer, Rainy & Winter) of sample collection were recorded at the time of fecal sample collection. The overall prevalence and variable-specific prevalence, along with 95% confidence intervals, were calculated using the 'tabpct' function from the R package "epiDisplay" (Mahmud *et al*., 2022). Univariable associations between cryptosporidiosis and explanatory variables, including age, sex, and season, were analyzed using logistic regression (Schlesselman, 1982) in R version 4.3.3 (The R Foundation for Statistical Computing, Austria).

Results

Cryptosporidium parvum oocysts measured 3–4 µm in diameter and were spherical. The organisms were localized along the gastrointestinal epithelium, specifically in the small intestine (Figure 1).

Cryptosporidiosis prevalence in cats from Mymensingh district was 8.3%, with oocyst counts per field ranging from 4 to 178 (Table 1). The prevalence was higher in young cats under 1 year of age (16.6%) compared to adult cats over 1 year (4.1%). Females showed a higher prevalence (10%) compared to males (6.2%). The infection was detected throughout the year except during the winter season. Prevalence was higher during the summer season (15.4%) compared to the rainy season (8.3%). However, none of the observed differences in prevalence by age, sex, or season were statistically significant (Table 2).

Post-mortem examinations revealed that the affected intestines appeared normal in size, shape, and overall appearance. Histopathological examination of the intestines in affected animals also did not reveal any remarkable changes.

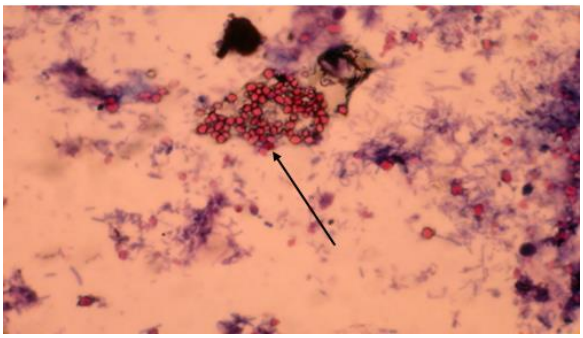

Fig. 1. *Cryptosporidium* sp. oocysts (arrow) in the feces of a cat.

Table 1. Overall prevalence and mean density of *Cryptosporidium* sp. in cats in Bangladesh

Fecal sample tested=36		Parasite burden
Positive	Prevalence (%) and 95% CI	Oocysts per focus (Range)
3	8.3 (2.2-23.6)	4-178

Table 2. Univariable association of feline cryptosporidiosis prevalence with age, sex, and season in Mymensingh district, Bangladesh

Variable	Category	Tested (Positive)	Prevalence (95% CI)	Odds ratio (95% CI)	P-value
Age (Year)					0.22
	Young (< 1)	12 (2)	16.6	4.6 (0.4, 56.7)	
	Adult (above 1)	24 (1)	4.2	Reference	
Sex					0.68
	Male	16 (1)	6.2	Reference	
	Female	20 (2)	10.0	1.67 (0.14, 20.23)	
Season					0.58
	Summer	13 (2)	15.4	2 (0.16, 25.4)	
	Rainy	12 (1)	8.3	Reference	
	Winter	11 (0)	0	Not included	

Discussion

The prevalence of *Cryptosporidium* sp. infection in this study was 8.3%, which aligns with findings reported by O'Callaghan *et al*. (2005) in Australia, Gennari *et al*. (1999) in Brazil, Nutter *et al*. (2004) in the USA, Huber *et al*. (2002) in Brazil, and Enemark *et al*. (2020) in Denmark, who recorded infection rates of 7%, 10%, 14.4%, 7%, and 6.7%, respectively. Slight differences in prevalence have been observed worldwide, such as the 2.3% infection rate reported by Li *et al*. (2019) in China and the 3% prevalence found by Köseoğlu *et al*. (2022) in Iran. These variations may stem from differences in climatic conditions, study methodologies, or diagnostic techniques.

Young cats (<1 year old) showed a significantly higher prevalence (16.6%) compared to adult cats (>1 year old), with younger cats being 4.6 times more likely to be infected. Similar observations were made by Robben *et al*. (2004) in the Netherlands, who reported higher prevalence rates in kittens and stray cats. McReynolds *et al*. (1999) in the USA noted a high seroprevalence (15.3%) in older cats (>10 years), while Rambozzi *et al*. (2007) in Italy found age to be a significant risk factor, with younger cats being more susceptible. The increased prevalence in younger cats may be due to their immature immune systems or greater exposure to sources of infection.

Females in this study were slightly more susceptible to infection (10 %) than males (6.25%), which contrasts with McReynolds *et al*. (1999), who reported higher prevalence in males (10.1%) than females (6.9%). However, this difference was not statistically significant. Rambozzi *et al*. (2007) and Tzannes *et al*. (2008) found no significant association between infection and sex. The slightly higher prevalence in females observed in this study could be attributed to physiological factors such as pregnancy or lactation, which may weaken immunity.

Seasonal variation in infection was observed, with the highest prevalence in summer (15.4%), followed by the rainy season (8.33%), and no infections recorded in winter. This is consistent with the findings of Tzannes *et al*. (2008), who observed higher prevalence in late autumn and early winter in the UK. In contrast, Hossain *et al.* (2019) reported the highest prevalence (81.4%) during the rainy season in Gambian children. Variations in infection prevalence across seasons may be influenced by climatic conditions, including temperature, humidity, and environmental factors, which can affect the survival and transmission of *Cryptosporidium* oocysts.

Post-mortem and histopathological examinations revealed no significant gross or microscopic changes in the intestines of affected cats,

indicating that *Cryptosporidium* sp. infection may not always result in severe pathological lesions.

Conclusion

The findings of this study confirm that *Cryptosporidium* sp. infection is a concern for domestic and stray cats in Bangladesh, with potential implications for feline health and zoonotic transmission to humans. While infection may not always cause severe pathology, its public health significance cannot be ignored. Developing sustainable, cost-effective strategies for prevention and control is crucial, and further investigations are needed to better understand the epidemiology and impact of *Cryptosporidium* sp. infection in humans and cats

Conflict of Interest

The authors declare no conflict of interest.

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