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Estimation of protozoal diversity among stray cats in Malaysia

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Abstract. *Abdulwahab MH, Al-Talib H. 2024. Estimation of protozoal diversity among stray cats in Malaysia. Biodiversitas 25: 2115-2120.* This cross sectional study was carried out to estimate the current prevalence and protozoa species in stray cats feces in Malaysia. A total of 320 fresh fecal samples were collected from S.I Home Shelter-Selangor, EJ CAT Home- Melaka, and KB-Cat Shelter - Kelantan in Malaysia. The collected fecal samples were concentrated for the presence of intestinal protozoa using a ready-made commercial Parasep tube floatation method. The fecal samples were stained with iodine and examined for protozoa by microscopic examination. The overall prevalence of intestinal protozoa in cat feces was 73.1% (n = 234). *Isospora felis* was most frequently detected (n = 145; 62%), followed by *Toxoplasma gondii* (n = 75; 32%), *Giardia intestinalis* (n = 9; 3.8%), *Entamoeba histolytica* (n = 9; 3.8%), and *Tritrichomonas foetus* (n = 6; 12.5%). Monoparasitism was detected in 65.8%, which was more frequent than polyparasitism (34.2%). Therefore, the local population is at risk of exposure to a wide range of zoonotic protozoa through environmental contamination with cat feces, and the current findings should be used to mitigate public health risks. Prevention and control measures need to be taken to reduce prevalence rates, especially in socioeconomically disadvantaged communities where animals live in close proximity to humans, where poor sanitation and overcrowding exist along with a lack of veterinary care and awareness of zoonotic diseases.

Keywords: Diversity index, intestinal protozoa, prevalence, stray cats, zoonotic diseases

INTRODUCTION

The bond between humans and their pets can have significant positive effects on emotional development, socialization, and physiological well-being (Ngui et al. 2014). Stray cats are important as potential reservoir hosts for several pathogens of medical and veterinary concern (Ebani et al. 2020). The diversity of intestinal parasites varies by geographic region, the presence of adequate veterinary care, and the type of cat population "domestic cats, cats in shelters, or stray cats" (Yang and Liang 2015). Parasitic diseases are more common in feral cats than in domestic cats, apparently due to a lack of treatment against parasites and greater reliance on wild animals as prey (Takeuchi-Storm et al. 2015). Protozoa are unicellular microorganisms belonging to the Protista kingdom, which also includes various other unicellular microorganisms (Negron 2020).

The most common protozoa found in cats are *Cryptosporidium* spp., *Giardia* spp., and *Tritrichomonas foetus* (Li et al. 2019). Cats are also an important host for *Toxoplasma gondii* and can excrete millions of oocysts after ingestion of a few tissue cysts (Tan et al. 2020). *Isospora* spp. are other protozoa and are among the numerous coccidia species that infect cats (Williams 2015). Cats are frequently infected with *Tritrichomonas foetus*, which colonizes their large intestine and causes persistent, uncontrollable diarrhea (Tolbert and Gookin 2016). The parasite spreads via the fecal-oral route, often via the sharing of litter boxes, contaminated water and food, and bowls can also be a possible source of infection. Fortunately, *Tritrichomonas foetus* is not transmissible from cats to humans (Suzuki et al. 2016). Cats infected with *Tritrichomonas foetus* may have diarrhea with a foul odor, mucus and blood, dribbling of stool, urinary urgency, and flatulence. Diagnosis is based on demonstrating of *Tritrichomonas foetus* in feces using light microscopy, protozoal culture, or PCR amplification using species-specific target genes (Bastos et al. 2019).

Detection of protozoal infections in cats is usually difficult because there are no or only nonspecific signs of disease; furthermore, cats with protozoal infections can present with a variety of clinical signs, ranging from mild gastroenteritis and failure to thrive to severe anemia and anorexia, depending on the protozoal species and its frequency (Marks 2016). However, *Cryptosporidium* spp., *Isospora* spp., and *Giardia* can cause severe gastroenteritis in cats with clinical signs (de Oliveira et al. 2021).

Parasitic diseases of pets include a wide range of arthropods, helminths, and protozoa. Protozoan parasites are transmitted between cats by direct contact, as in the case of *Tritrichomonas foetus*, or by exposure to resistant protozoan stages in the environment, as in the case of *Giardia felis*, *Cryptosporidium parvum*, and *Isospora* spp. Other methods of transmission include ingestion of other hosts containing resistant protozoan stages, as in the case of *Sarcocystis* spp. and *Toxoplasma gondii*. The final method of transmission is by a blood-feeding arthropod vector, as in *Leishmania* spp., *Trypanosoma* spp., and Babesia (Pereira et al. 2016).

Previous study has shown that cats under 7 months of age, in particular, are more susceptible to protozoal diseases such as *Toxocara cati* and species of *Cystoisospora* (Cossío et al. 2021). Cats play an important role in the transmission of zoonotic diseases to humans. Transmission of parasitic

protozoa to humans occurs through close contact with parasitic animals or contact with a contaminated environment (Al-Kubaisy et al. 2014).

Giardia spp. isolates from humans and cats are not different. Transmission of *Giardia* spp. from a cat to humans has not been documented. However, all cats infected with *Giardia* spp. should be considered potentially zoonotic. *Toxoplasma gondii* is one of the most common parasites of cats that can infect humans worldwide (Karimi et al. 2022). *Toxoplasma gondii* is transmitted to humans by accidental oral ingestion of food or water contaminated with infectious oocysts or by consumption of raw or inadequately cooked meat containing bradyzoite tissue cysts; also, by transplacental transmission to the foetus during primary infection in a pregnant woman. *Toxoplasma gondii* is of major public health concern, especially to pregnant women and immunocompromised individuals, and causes congenital malformations and abortions of pregnancies (Chemoh et al. 2016).

The diversity and prevalence of intestinal protozoan infections in stray cats are poorly understood in Malaysia. Most previous studies on intestinal protozoa have only included domestic cats. Therefore, this study was conducted to determine the diversity and prevalence of intestinal protozoa in stray cats in Malaysia testing fecal samples.

MATERIALS AND METHODS

Fecal samples collection

This cross-sectional study was conducted to assess protozoal diversity among stray cats in Malaysia. A total of 320 fresh cat fecal samples were collected from S.I Home Shelter-Selangor, EJ CAT Home- Melaka, and KB-Cat Shelter - Kelantan. It was assumed that the fecal samples were from different individual cats. The fecal samples were collected with permission from the shelter owner and assistance from staff. However, the age and history of the cats were not known because they were brought to the shelter from different circumstances and situations. Approximately 3 to 4 grams of preferably moist and shiny fresh feces were collected and placed in a clean, wide-mouthed container with a tight-fitting lid and sealed in a plastic bag for transport to the laboratory. Fecal samples were fixed and preserved in 10% formalin and stored in a refrigerator at 4°C until use.

Fecal concentration

Collected fecal samples were concentrated using the MINI PARASEP® Tube (Apacor, Berkshire, England). In the mixing chamber, 1 g of the fecal sample was added along with one drop of Triton X solution and 1 mL of ethyl acetate and mixed thoroughly. The samples were then centrifuged at 1200 g for three minutes. Then all the supernatant was poured off and one drop of the sediment was pipetted for slide preparation (Fecal Parasite Concentrators 2023). Two staining methods were used, including:

Trichrome staining

A fecal sample previously fixed with polyvinyl alcohol on a slide was placed in 70% ethanol plus iodine for 10 minutes. Then the slide was placed twice in 70% ethanol for 5 minutes and 3 minutes. After that, the slide was stained with trichrome for 10 minutes and then decolorized in 90% ethanol plus acetic acid for 2 seconds. Finally, the slide was rinsed several times in 100% ethanol and mounted with a coverslip using a mounting medium (Datta et al. 2024).

Modified Ziehl-Neelsen acid-fast staining (Cold Kinyoun)

The Cold Kinyoun technique with modification was performed for the detection of Cryptosporidium as described by Henriksen and Pohlenz (1981). Two slides per sample were prepared and examined using 400x and 1000X under oil immersion magnification using a light microscope (Olympus BX61, Japan). Identification and documentation of intestinal protozoa was based on the morphology and size of each species according to McHardy et al. (2014). Each specimen was examined, and each slide was considered positive if at least three protozoan cysts/oocysts, trophozoites were observed.

Data analysis

All microscopical examination were performed at least in triplicate. Diversity index such as Shanon-Wiener, Simpson, and Dominance Index were calculated online using https://www.alyoung.com/labs/biodiversity calculator.html. Data represent the mean of three replicates±Standard Deviation (SD). Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 28.0 (SPSS Inc., Chicago, US).

RESULTS AND DISCUSSION

Out of 320 samples examined, 234 were positive, representing an overall prevalence of 73.1% of intestinal protozoa detected in cat feces. Five different protozoa were detected in cat feces, including *Toxoplasma gondii*, *Isospora felis*, *Giardia intestinalis*, *Entamoeba histolytica*, and *Tritrichomonas foetus*. The highest rate of protozoa detected was *Isospora felis* (62%), followed by *Toxoplasma gondii* (32%), *Entamoeba histolytica* (3.8%), *Giardia intestinalis* (3.8%), and *Tritrichomonas foetus* (2.5%) (Table 1). Among individual protozoa in cat feces, *Isospora felis* had the highest rate (77.3%), followed by *Toxoplasma gondii* (18.8%) and *Entamoeba histolytica* (3.9%), as shown in Table 2.

This study showed that single protozoa from fecal samples were more abundant (65.8%) than mixed protozoa (34.2%). Among mixed protozoa, both *Toxoplasma gondii* and *Isospora felis* were most abundant in cat feces (19%), followed by *Isospora felis* and *Entamoeba histolytica* (6.25%) (Table 3, Figure 1). This study revealed limited diversity of cat feces protozoa in Malaysia as a whole and in Selangor, Melaka, and Kota Bharu state-wise (Tables 4 and 5).

Discussion

Parasitic diseases continue to be a severe burden on animal and human populations in tropical and subtropical regions of the world (Short et al. 2017). However, one of the major problems in controlling parasitic diseases is the low level of awareness among livestock owners and the varying perception of their responsibility to respond appropriately to this situation (Alho et al. 2018). A previous study by Pereira et al. (2016) showed that 44% of people were unaware of the possible transmission of parasites from their pets to themselves. Cats can serve as essential hosts for zoonotic diseases that affect humans, although their owners often consider them part of the family (Keesing and Ostfeld 2021). Many types of parasitic infestations in Malaysian animals include stray cats and domestic cats. These diseases include filariae in domestic cats and stray cats, *Dipylidium caninum* in rural dogs, and a variety of other animal parasites that have been detected in soil and fecal samples from dogs and cats (Nguyen et al. 2021). This underlines the importance of understanding and keeping track of parasitic diseases in domestic and stray animals to stop the spread of zoonoses and protect public health. Protozoal infections in stray cats can be a significant problem as these infections can affect the cats and potentially be transmitted to humans. Protozoal infections in cats can pose a zoonotic risk, i.e., they can be transmitted from cats to humans. The three most common protozoal diseases that can affect both cats and humans are cryptosporidiosis, giardiasis and toxoplasmosis. Stray cats with protozoal infections showed various signs such as severe diarrhea, weight loss, loss of appetite, difficulty defecating, dehydration, vomiting, and the presence of mucus or oocysts in the feces (Adhikari et al. 2023). Stray cats showed a higher seroprevalence and molecular prevalence of intestinal protozoa than domestic cats, but there was no significant difference between the two groups (Tan et al. 2020).

This study was conducted to investigate the diversity and prevalence of protozoa in cat feces using the commercial MINI PARASEP® Tube fecal sedimentation method. This study found a high prevalence (73.1%) of protozoa in cat feces from three animal shelters in Malaysia. However, since there are no data or reports on the prevalence of protozoa in cat feces in Malaysia, it is difficult to compare this study's prevalence with that of previous studies in Malaysia.

Table 1. Common protozoa detected in cat feces.

Protozoa	n	% a	% b
Toxoplasma gondii	75	32	23.5
Isospora felis	145	62	45.3
Giardia intestinalis	9	3.8	2.8
Entamoeba histolytica	9	3.8	2.8
Tritrichomonas foetus	6	2.5	1.9

Note: a Prevalence was calculated based on total number of infected cats for each species (n) divided by total number of infected animals (234 cats) x 100%; b Prevalence was calculated based on total number of infected cats for each species (n) divided by total number of cats sampled (320 cats) x 100%

Table 2. Single protozoa in cat feces

Protozoa	n	%
Toxoplasma gondii	29	18.8
Isospora felis	119	77.3
Giardia intestinalis	0	0
Entamoeba histolytica	6	3.9
Tritrichomonas foetus	0	0
Total	154	100

Note: Prevalence was calculated based on total number of infected cats for each species (n) divided by total number of cats with single infection (154 cats)

Table 3. Mixed protozoa in cat feces

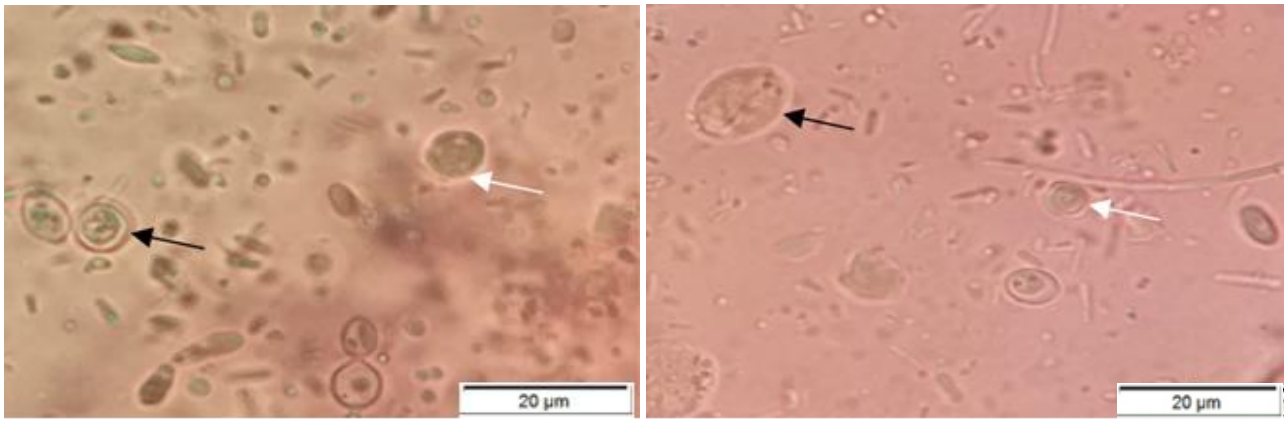
Protozoa	n	%
Isospora felis and *Entamoeba histolytica*	5	6.25
Isospora felis and *Giardia intestinalis*	4	5
Toxoplasma gondii, *Isospora felis*, *Giardia intestinalis*, and *Tritrichomonas*	3	3.75
Toxoplasma gondii and *Isospora felis*	60	75
Toxoplasma gondii, *Isospora felis*, *Giardia intestinalis*, and *Entamoeba histolytica*	3	3.75
Toxoplasma gondii, *Isospora felis*, and *Giardia intestinalis*	3	3.75
Toxoplasma gondii, *Isospora felis*, and *Tritrichomonas*	2	2.5
Total	80	100

Note: Prevalence was calculated based on number of cats with mixed species (n) divided by total number of cats with mixed infection (80 cats)

Table 4. Diversity indices for protozoa detected in cat feces within three states in Malaysia

Species	Selangor				Melaka				Kota Bharu			
	No.	Pi	Ln Pi	Pi * InPI	No.	Pi	In Pi	Pi * InPI	No.	Pi	In Pi	Pi * InPI
Toxoplasma gondii	30	0.28	-1.25	-0.35	17	0.31	-1.15	-0.36	28	0.32	-1.11	-0.36
Isospora felis	63	0.6	-0.53	-0.30	30	0.55	-0.58	-0.32	52	0.61	-0.49	-0.30
Giardia intestinalis	5	0.04	-3.04	-0.14	3	0.05	-2.89	-0.16	1	0.01	-4.44	-0.05
Entamoeba histolytica	4	0.03	-3.26	-0.12	3	0.05	-2.89	-0.16	2	0.02	-3.74	-0.08
Tritrichomonas foetus	3	0.02	-3.55	-0.10	1	0.01	-3.98	-0.07	2	0.02	-3.74	-0.08
Total	105	1		-1.04	54	1		-1.09	85	1		-0.89
Shanon-Wiener Index			1.04				1.09				0.89	
Simpson Index			0.44				0.40				0.47	
Dominance Index			0.55				0.59				0.52	

Note: Pi proportion of each species (relative abundance), LnPi natural logarithm of Pi



A **B**

Figure 1. Smear from cat feces (×100 objective) revealed mixed intestinal protozoa. A. *Isospora felis* (*black arrow*) and *Toxoplasma gondii* (*white arrow*); B. *Giardia intestinalis* (*black arrow*) and *Toxoplasma gondii* (*white arrow*)

Table 5. Overall diversity indices for protozoa detected in cat feces in Malaysia

Species	No.	Pi	In (Pi)	Pi*In (Pi)
Toxoplasma gondii	75	0.30	-1.17	-0.36
Isospora felis	145	0.59	-0.52	-0.30
Giardia intestinalis	9	0.03	-3.29	-0.12
Entamoeba histolytica	9	0.03	-3.29	-0.12
Tritrichomonas foetus	6	0.02	-3.70	-0.09
Total	244	1	0	-1.006
Shanon-Wiener Index				1.006
Simpson Index				0.449
Dominance Index				0.551

Note: Pi proportion of each species (relative abundance), LnPi natural logarithm of Pi

Immunocompromised cats are at high risk of contracting intestinal protozoal infections (Lebbad et al. 2021). The infection is caused by the ingestion of food or water contaminated with protozoa and leads to severe diarrhea and dehydration. Diagnosis of cat intestinal protozoal infection includes a physical examination and a fecal smear to detect oocysts. Treatment may include antibiotics and, in severe cases, fluid replacement therapy to combat dehydration (Ahmed 2023).

Previous studies have shown that cat fecal samples from the Klang Valley in Malaysia had a high seroprevalence of 5.5% *T. gondii* DNA. Although there was no significant difference between the two groups, stray cats had a higher seroprevalence and molecular prevalence of *T. gondii*. Furthermore, the research showed that a small number of Malaysian stray cats and domestic cats are releasing *T. gondii* oocysts into the environment, which could pose a public health risk (Tan et al. 2020). Another study demonstrated the overall prevalence of gastrointestinal protozoa in the Klang Valley cat population was 10.4% for *Toxoplasma gondii*, 7.9% for *Giardia intestinalis* and 50% for *Cystoisospora* spp. (Tan 2019).

High rates of protozoa in cat feces have been found worldwide, such as 42% in Iran and 41.39% in China (Esmaeilzadeh et al. 2009; Yang and Liang 2015). Our study found that *Isospora felis* had the highest rate among the other protozoa in cat feces (62%), which is consistent with the results of a previous study conducted in China (41%) (Yang and Liang 2015). In addition, a study conducted in North America, Alaska, and Hawaii found that the prevalence of *Isospora* spp. in cats ranged from 3% to 36%. Isospora infection in cats is usually asymptomatic. However, under certain conditions, such as in a crowded environment, a cat infected with *Isospora* spp. may exhibit diarrhea, vomiting, and loss of appetite. However, *Isospora* in cats cannot cause disease in humans. This is because all *Isospora* spp. are host-specific (Scorza et al. 2021).

Cats infected with *Toxoplasma gondii* may not show signs of illness, but if they do, symptoms may include fever, weight loss, eye problems, and neurologic problems (Rahimi et al. 2015). Cats can shed the parasite for short periods, mainly in their feces, but they are not a significant source of infection for humans. It is unlikely that one will become infected with the parasite by touching an infected cat or through bites/scratches. Indoor cats that do not hunt or eat raw meat are less likely to be infected with *Toxoplasma gondii*. Diagnosis of cat toxoplasmosis involves measuring antibodies in the blood. Cat toxoplasmosis is treated with antibiotics such as clindamycin and anti-inflammatory corticosteroids. Pregnant women and immunocompromised individuals have a higher risk of complications from *Toxoplasma gondii* exposure (Maqsood et al. 2021).

In this study, a high rate of *Toxoplasma gondii* (32%) was found in cat feces, which was higher than in a previous study in Klang Valley, Malaysia, where a prevalence (10.5%) of *T. gondii* DNA was detected in cat fecal samples, indicating a higher shedding of *T. gondii* oocysts (Tan et al. 2020). The differences in the prevalence of *T. gondii* between our study and previous studies may be due to the use of different techniques. However, this is higher than previous studies in Czechoslovakia (17%), Brazil (20%), and Costa Rica (23%). On the contrary, higher rates of were reported in Turkey (40%) and Egypt (41%) (CAPC 2014). The high percentage of feline toxoplasmosis in some areas may be due to high humidity and temperate

climate, lack of treatment for feline toxoplasmosis, and large numbers of cats. Another study conducted in Virginia, United States, found that only 6% of the cats studied actively excreted *T. gondii* oocysts in their feces (Lilly and Wortham 2013). Another study in Brazil found that *T. gondii* was detected in 58 domestic cats from 51 households suggesting that pets are likely a risk factor for transmission of *Toxoplasma gondii* to humans, although socioeconomic and environmental factors may also play an important role in disease transmission (Dubey et al. 2004). Zoonotic transmission of toxoplasmosis depends on the presence of infected animals, behavior, or owners' knowledge and understanding of prevention measures.

The third most common protozoa in cat feces is *Giardia* spp. In this study, the prevalence of *G. intestinalis* was 3.8%, but it may vary depending on the environment according to Cornell Feline Health Centre Cats Brochure. A previous study in Germany showed that a total of 60 fecal samples from cats were infected with *Giardia* spp. (Pallant et al. 2015). Other study showed that the prevalence of *Giardia* in cat feces varied worldwide, which could be due to differences in sex, age, breed, and symptoms of cats (Bouzid et al. 2015). Another study using ELISA to detect *Giardia* spp. in fecal samples from domestic cats from Romania found that 28% of fecal samples had antigens of *Giardia* spp. (Mircean et al. 2011). The worldwide variation in parasitic protozoan detection rates in different studies could be due to differences in detection methods (Al-Talib et al. 2019). It is well known that microscopy compares poorly with other detection methods. In addition, microscopy is not as standardized as the other methods, and the concentration steps of the preparations can affect the sensitivity of the technique. However, a previous study found that the use of the zinc sulfate flotation technique followed by microscopy can improve the detection rate of intestinal protozoa (Potes-Morales and del Pilar Crespo-Ortiz 2023).

In this study, we also found the highest rate of mixed infections due to *Toxoplasma gondii* and *Isospora felis* being 75%. Mixed infections of cats with *Giardia* and other protozoa were also noted in this study. Our results are consistent with a previous study in Germany reporting mixed infections with *Giardia* cysts, *Toxoplasma gondii* and *Cystoisospora* spp. (Pallant et al. 2015). Giardiasis in cats can cause symptoms such as vomiting, weight loss, flatulence, and severe, watery diarrhea (Adhikari et al. 2023). *Giardia* is extremely contagious and can infect humans, dogs, cats, and other animals (Godínez-Galaz et al. 2019). The fecal-oral pathway spreads *Giardia*; cysts that are released in the feces become infectious as soon as they are excreted. These cysts can be ingested by cats directly from an infected host, via contaminated food or water or via their environment. Diagnosis of *Giardia* infections has evolved from microscopic examination of feces to immunologic testing (Ahmed 2023). Treatment of *Giardia* infections in cats is not always effective; reinfections are common.

The prevalence of *Entamoeba histolytica* in the current study was quite low, yet it is important to study it because amebiasis is the third leading cause of death in humans due to parasitic infection after malaria and schistosomiasis. However, a previous study in the Malaysian states of Selangor and Pahang showed that the most common protozoa detected in cat feces were *Entamoeba* spp. and *G. intestinalis* (Ngui et al. 2014). Another study conducted in Berlin found that only 1 of 13 cats excreted cysts of *Entamoeba* spp. in their feces. The low prevalence of *Entamoeba* spp. could be because every cat with diarrhea is treated immediately, or it could be due to the low sensitivity of the microscopic method. However, colonoscopy and tissue scraping, or biopsy of the affected colon tissue are more accurate in detecting *Entamoeba* spp. than fecal examination (Fleming et al. 2015). In addition, repeat testing is required when feces are identified because cysts of *Entamoeba* spp. may be periodically excreted with feces. It is important to reduce the prevalence of protozoa in cats as it can have negative public health implications. Among the negative effects that should be prevented are zoonotic diseases. Although some of the protozoa in cats are not pathogenic or species-specific (they cannot be transmitted to humans), it is our responsibility to treat these infections in cats because cats are the primary companions of humans and have a close relationship with communities.

In conclusion, the prevalence of stray cats shedding intestinal protozoa such as *Toxoplasma gondii* and *Isospora* spp. in Malaysia is still high. It is, therefore, the veterinary profession must not forget this important aspect of animal welfare. Further research is needed to investigate the genetic diversity of protozoa in humans, which may also be present in stray cats. Nonetheless, these infections must be detected and treated before they lead to severe disease or spread to other domestic cats or even their owners.

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