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Zoonotic gastrointestinal nematodes in pet cats in Yogyakarta (Indonesia) and their susceptibility to anthelmintics

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Abstract. *Widiyono I, Rusmihayati, Purnamaningsih H, Sahara A. 2023. Zoonotic gastrointestinal nematodes in pet cats in Yogyakarta (Indonesia) and their susceptibility to anthelmintics. Biodiversitas 24: 3332-3337.* Domestic cats (*Felis catus* Linnaeus, 1758), which live close to humans, can host some zoonotic nematodes. Therefore, a study on gastrointestinal nematodes in domestic cats and their anthelmintic resistance is essential for controlling and preventing zoonotic nematodiasis. Our goal was to investigate the diversity of gastrointestinal nematodes in pet cats in Yogyakarta and their susceptibility to anthelmintics. One hundred seventy-four (174) out of 215 cat patients (80.93%) presented at the animal clinic of the Department of Internal Medicine, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, and suspected gastrointestinal parasite infections suffered from nematodiasis. Ninety-eight (98) animals suffering from moderate to severe gastrointestinal nematodiasis with Egg Per Gram feces (EPG) >300 were included into nematode identification, clinical analyses, and evaluation of nematode susceptibility to anthelmintics. The animals were divided in three treatment groups, namely pyrantel pamoate at the dose of 5 mg/kg BW orally on D0 (OP), subcutaneous injection of ivermectin at the dose of 200 µg/kg BW on day 0 (SCI), and no medication as a Control (C). Fecal samples were collected on day 0 and 10 for parasitological examinations. The results showed that pet cats were infected with *Toxocara* sp. and *Ancylostoma* sp., with the incidence of single infection by *Toxocara* sp., *Ancylostoma* sp., and mixed infection at 50%, 36.73%, and 13.27%, respectively. The animals were clinically healthy or showing clinical signs, e.g., anorexia, diarrhea, vomiting, and dull hair. Pyrantel pamoate and ivermectin showed >98% efficacy against these nematodes. In conclusion, there were two zoonotic gastrointestinal nematodes in pet cats in Yogyakarta, namely *Ancylostoma* and *Toxocara*, which still showed high susceptibility to pyrantel pamoate and ivermectin.

Keywords: Ancylostoma, anthelmintic resistance, clinical signs, efficacy, hematology, Toxocara, zoonoses

INTRODUCTION

Gastrointestinal nematodes are known to be agents that can potentially interfere with animal health and the general welfare of society in various parts of the world. Gastrointestinal nematode infection is a significant obstacle in animal health and public health (Beugnet et al. 2014; de Mello et al. 2022; Martínez-Sotelo et al. 2022; Nath et al. 2022; Tadege et al. 2022; Nath et al. 2023). Cats (*Felis catus* Linnaeus, 1758) from private households, kennels, and feral cats can be infected by gastrointestinal nematodes, i.e. *Ancylostoma*, *Uncinaria*, *Toxocara*, *Toxascaris*, *Physaloptera*, *Strongyloides*, and *Capillaria* (Wright et al. 2016; Dybing et al. 2018; Darabi et al. 2021; Adhikari et al. 2023). Nematodes infections have been reported in cats kept indoors without outdoor access or kept with outdoor access or free-living (Khademvatan et al. 2014; Wright et al. 2016; Pereira et al. 2017; Dybing et al. 2018; Wulcan et al. 2019; Darabi et al. 2021; Adhikari et al. 2023) and resulted in host health problems, such as a dull coat, vomiting, diarrhea, loss of appetite, a potbellied appearance, anemia, gastroenteritis, stomach rupture, coughing, and pneumonia (Epe 2009; Calista et al. 2019; Alves et al. 2021; Lima and Del Piero 2021; Ursache et al. 2021). In addition, other issues that are important to consider are the presence of potentially zoonotic nematodes and environmental/soil pollution (Bojar and Kłapeć 2018; Nava et al. 2020; Stafford et al. 2020; de Mello et al. 2022). Zoonotic nematodes, i.e. *Toxocara*, *Ancylostoma*, and *Capillaria*, are transmitted to humans via the mouth (food, water) or skin invasion and without the involvement of intermediate hosts or vectors (Otranto and Deplazes 2019; Traub et al. 2021). In humans, these zoonotic gastrointestinal nematodes can cause Cutaneous Larva Migrans (CLM) and Visceral Larva Migrans (VLM) (Del Giudice et al. 2019; Otranto and Deplazes 2019; Özbakış and Doğanay 2020; Traub et al. 2021).

Until now, one of the efforts often used to overcome the problem of helminth infections is deworming drugs. These medications have been common in achieving effective control programs against gastrointestinal nematode infections for nearly four decades. Benzimidazoles, imido thiazoles-tetrahydro pyrimidines, and ivermectin are the main classes of anthelmintics used to control gastrointestinal nematodes in large and small animals (Abongwa et al. 2017). These anthelmintics are often the choice because of their broad spectrum nematocidal activity and low toxicity, and thiabendazole became a pioneer since its introduction in 1961. However, frequent use or subtherapeutic doses significantly contribute to the development of resistance to anthelmintics, which in turn poses a serious threat to domestic animals and the health of both animals and

humans (Reviere and Papich 2017; Kebede 2019; Kitchen et al. 2019; Zekarias and Toka 2019; Fissiha and Kinde 2021).

Resistance was first reported to phenothiazines in the 1950s, then benzimidazoles in the 1960s, imido thiazoles-tetrahydro pyrimidines in the 1970s, and ivermectin in the 1980s. Recently, the resistance of gastrointestinal nematodes to anthelmintics in animals has been reported worldwide, and single and multiple anthelmintic resistance is of most significant concern worldwide (Castro et al. 2019; Kitchen et al. 2019; Zekarias and Toka 2019; Fissiha and Kinde 2021; Castro et al. 2021; O'Halloran 2021). Some anthelmintic resistance poses a serious threat to animals and society and is very prevalent in countries such as the United States (Castro et al. 2019, 2021), China (Wang et al. 2017), and Bangladesh (Nath et al. 2023).

Nowadays, more and more people keep cats as pets in Indonesia (Bayu 2021; Nurhayati-Wolff 2022). Thus, there needs to be a severe concern for monitoring gastrointestinal nematode anthelmintic resistance in the Indonesian region, especially Yogyakarta. Detecting the resistance of gastrointestinal nematode worms to anthelmintics at low levels as early as possible is considered very important to minimize the abandonment of resistant parasites. Accurate and timely detection of anthelmintic resistance is essential for preventing and controlling the spread of anthelmintic resistance and ultimately controlling parasites of broad economic and public health importance. However, to the best of the authors' knowledge, there needs to be more attention to assessing the efficacy of anthelmintics against nematode infestations and detecting the presence of anthelmintic resistance in pets in Yogyakarta. Therefore, this study aimed to investigate the diversity of gastrointestinal nematodes in pet cats in Yogyakarta and their susceptibility to anthelmintics currently available. The results of this study could be necessary for the considerations in the treatment, eradication, and control of gastrointestinal nematode worm infections.

MATERIALS AND METHODS

Ethical approval and informed consent

The Ethical Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (0123/EC-FKH/Int./2022) reviewed and approved the study. Cat owners were informed about the study aims, i.e., gastrointestinal nematodes investigation and evaluation of their anthelmintics susceptibility in domestic cats.

Animals and methods

Pet cats presented to the Department of Internal Medicine, Universitas Gadjah Mada's animal clinic, for medical examination and treatment from April through November 2022 and assessment of suspected gastrointestinal parasite infections (215 cat patients) were used in this study. The cat owners completed a survey about their cat, i.e., age, sex, breed, feeding practice, keeping methods, number of cats kept in the family, and defecation habits. The animals included in the recent study were 4-48 months old, male or female, local or mix breed, fed commercial or homemade feed, given the opportunity to defecate in a litterbox or outdoors, and kept indoors or free roaming around the keeper's house in small (up to 6 animals) or large (more than ten animals) group in Yogyakarta, Indonesia, for over three months. This area is located between 07°15'24"-07°49'26"S and 110°24'19"-110°28'53"E, at an altitude between 100-499 m.a.s.l., and has a tropical climate with two seasons of dry and rainy seasons. Furthermore, only the animals suffering from gastrointestinal nematode infection with a total number of nematode Eggs Per Gram of feces (EPG) of more than 300 were included in nematode identification, clinical analyses, and evaluation of nematode susceptibility to anthelmintics commonly used in Yogyakarta. Individual data, maintenance management, fecal and venous blood sample collection, and clinical examinations in each animal were carried out on day 0 (D0) or pre-treatment. The Body Condition Score (BCS) of the animal was assessed by an experienced veterinarian according to a 9-point BCS system (1 emaciated - 9 grossly obese) (Sapowicz et al. 2016). The individual fecal score was estimated using a visual scoring chart with a scoring range of 1 (very hard, dry) - 7 (watery, no texture) scale (Felten et al. 2022). The selected animals were divided into groups depending on the medical treatment, which were oral treatment of pyrantel pamoate at a dose of 5 mg/kg BW (OP), subcutaneous injection of ivermectin with a dose of 200 µg/kg BW SC (SCI), and no medication as Control (C). After clinical examination and samples and data collection on D0, the animals in group OP and SCI were treated with pyrantel pamoate and ivermectin, respectively. The animals in the control group remained untreated. On D10, after the anthelmintic treatment, fecal sample collections were conducted again for post-treatment parasitic examination. Fecal examinations were carried out for parasite identification and determination of the total number of nematode Eggs Per Gram of feces (EPG). The parasitological examination was carried out using the floating and McMaster technique (Ntampaka et al. 2021). The blood samples were used for hematologic and plasma protein examination. Hematological parameters were analyzed using standard methods, and plasma proteins were determined using a refractometer, as described by Fonteque et al. (2016). The efficacy and resistance to anthelmintics were assessed based on the individual Fecal Egg Count Reduction Test (iFECRT%) and the control one (cFECRT%) (Coles et al. 2006; Cabaret et al. 2004). Anthelmintic resistance was demonstrated when the iFECRT% and cFECRT% were lower than 95%, and the lower value of the 95% Confidence Interval (CI) was lower than 90%.

Statistical analyses

A Chi-square test was conducted to compare the likelihood of the association between risk variables (age, sex, breed, feeding practice, keeping methods, number of cats kept in the family, and defecation habits, clinical signs) and nematode infection. The influence of nematode infection on hematologic and blood chemistry parameter values and fecal scores was analyzed using the Kruskal-Wallis test. The difference between means was assessed using post-hoc test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Gastrointestinal nematode infestation in pet cats

A total of 174 out of 215 (80.93%) pet cats kept in the household in Yogyakarta were presented to the animal clinic of the Department of Internal Medicine of the Faculty of Veterinary Medicine, Universitas Gadjah Mada for medical examination and treatment from April through November 2022 and assessment of suspected gastrointestinal parasite infections suffered from nematodiasis. Ninety eight (98) animals suffering from gastrointestinal nematode infestation with an EPG of more than 300 were included in nematode identification, clinical examinations, and evaluation of nematode susceptibility. The results of the parasitological examinations revealed that 49 out of 98 cats examined (50%) suffered from toxocariasis, 36 (36.73%) suffered from ancylostomiasis, and 13 (13.27%) suffered from mix-infestation of *Toxocara* and *Ancylostoma*.

The biological data and management of pet cats in Yogyakarta with nematodiasis are presented in Table 1. Incidence of infection was found in young and adult animals of both sex groups without any predilection for age and breed differences (P>0.05). Furthermore, nematodiasis occurred in animals kept in small (6 heads or fewer per family) and large group (10-45 heads per family) with significantly higher predilection in the large group (P<0.05). The incidence of ancylostomiasis and toxocariasis in animals fed with commercial or homemade feed, kept indoors and free to roam outside the house, and defecating in a litter box and outdoors were similar (P>0.05).

Table 2 presents the clinical findings that could be present in some parts of the animals with nematodiasis, i.e., diarrhea (average fecal consistency score of 4.5-5.9), dull hair (40.82-55.56%), anorexia (2.78-23.08%), and vomiting (2.78-15.38%). Anorexia, vomiting, and higher pulse rate and rectal temperature were more frequently observed in animals with mixed infestation than that with a single infection of Ancylostoma. However, most animals had an ideal body condition, namely an overall body condition score of 4.56-5.57. The values of physiological, blood chemistry (plasma protein), and hematological parameters were still in the physiological range, except for the higher leucocyte count in cats with a mix-infection of *Ancylostoma* and *Toxocara* (21157.69±9000.30 cell/µL).

Anthelmintic resistance test of *Ancylostoma* and *Toxocara* in pet cats in Yogyakarta

Before this study began, animals in the OP, SCI, and C groups were observed to have severe natural nematode infections with EPGs at the average of 2031.58, 1016.67, and 970.83 (Table 3). Administration of the drugs pyrantel pamoate and ivermectin resulted in a significant decrease in EPG in the OP and SCI groups to 16.67 and 4.76 in D10, respectively. In contrast, the EPG of group C (untreated) did not show any significant change, namely 953.33. The iFECRT by the oral treatment with pyrantel pamoate and subcutaneous injection of ivermectin was 99.54±1.24% (95% confidence interval: 99.08%-100%) and 99.75 ± 0.96 (95% confidence interval: 99.40%-100%), meanwhile the cFECRT was 98.48% and 99.57%, respectively (Table 3).

Discussion

The percentage of nematodiasis cases in pet cats in Yogyakarta from April to December 2022 was higher than that reported in the Smilevet clinic in Jakarta for one year (January 2020-January 2021), namely 1.09% (Natasya et al. 2021). *Toxocara* and *Ancylostoma* were the main gastrointestinal parasites that infected the pet cats. It is in line with the findings reported by Purnama et al. (2019) that *Toxocara* and *Ancylostoma* are the dominant causes of nematodiasis in cats kept in shelters in Surabaya, namely 57.14% caused by *Toxocara* and 42.86% caused by *Ancylostoma*. Furthermore, Rabbani et al. (2020) also reported that nematodiasis incidence in pet and feral cats in Lumajang, East Java, Indonesia was 65% and 76.19% caused by *Toxocara* and 35% and 23.81% caused by *Ancylostoma*, respectively. Ursache et al. (2021) also found that the most frequent nematodes identified in domestic cats in Romania are *Toxocara* (40.2%) and *Ancylostoma* (3.7%) in single or mixed infections. Martínez-Sotelo et al. (2022) also reported that *Ascaris*, *Toxacara*, and *Ancylostoma*, are the predominant zoonotic worms detected in free-ranging domestic dogs (*Canis lupus* subsp. *familiaris* Linnaeus, 1758).

Table 1. Biological data and management of pet cats with nematodiasis in Yogyakarta, Indonesia (n = 98)

Parameter		Incidence of nematodiasis (%)			x^2/P value
		Ancylostomiasis	Toxocariasis	Ancylostomiasis and toxocariasis	
Age	<1 year (n=22, 22/98)	31.81 (7)	40.91 (9)	27.27 (6)	4.852/0.088
	>1 year (n=76, 76/98)	38.16 (29)	52.63 (40)	9.21 (7)	
Sex	Male (n=48)	29.17 (14)	62.5 (30)	8.33 (4)	6.132/0.047
	Female (n=50)	44 (22)	38 (19)	18 (9)	
Breed	Local (n=79)	31.64 (25)	55.69 (44)	12.66 (10)	5.630/0.060
	Mix (n=19)	57.89 (11)	26.32 (5)	5.79 (3)	
Number of animals kept	~ 6 animals (n=68)	22.06 (15)	58.82 (40)	19.12 (13)	22.218/0.000
	10-45 animals (n=30)	70 (21)	30 (9)	0	
Feed	Commercial (n=82)	34.15 (28)	50 (41)	15.85 (13)	3.453/0.178
	Homemade (n=16)	50 (8)	50 (8)	0	
Keeping	Indoor (n=66)	39.39 (26)	51.52 (34)	9.09 (6)	3.137/0.208
	Outdoor (n=32)	31.25 (10)	46.88 (15)	21.88 (7)	
Defecation	Litter box (n=84)	38.10 (32)	47.62 (40)	14.29 (12)	1.425/0.491
	Outdoors (n=14)	28.57 (4)	64.29 (9)	7.14 (1)	

Table 2. Clinical and laboratory findings in the pet cats with ancylostomiasis and toxocariasis in Yogyakarta, Indonesia

Clinical and laboratory findings	Infestation of gastrointestinal nematode			x^2/*P* value
	Ancylostomiasis (n=36)	Toxocariasis (n=49)	Ancylostomiasis and toxocariasis (n=13)	
Anorexia	1/36 (2.78%)	10/49 (20.41%)	3/13 (23.08%)	6.214/0.045
Vomiting	1/36 (2.78%)	4/49 (8.16%)	2/13 (15.38%)	2.442/0.295
Dull hair	20/36 (55.56%)	20/49 (40.82%)	6/13 (46.15%)	1.814/0.404
Fecal consistency (a 1-7 scale)	4.47 ± 1.30[a]	5.22 ± 1.23[b]	5.92 ± 1.26[b]	0.001
BCS (a 1-9 scale)	4.56 ± 1.21	5.57 ± 1.32	4.86 ± 0.90	0.079
Rectal temperature (°C)	38.70 ± 0.56[a]	38.49 ± 0.41[a]	38.94 ± 0.21[b]	0.018
Pulse rate (x/min)	114.52 ± 23.27[a]	126.53 ± 20.26[b]	132.86 ± 15.25[b]	0.007
Respiration rate (x/min)	43.65 ± 8.80[a]	48.12 ± 13.80[b]	37.14 ± 4.30[c]	0.001
Erythrocyte (10^6 cell/µL)	6.19 ± 1.09	6.37 ± 1.54	5.96 ± 1.24	0.611
Leucocyte (cell/µL)	16270.83 ± 9481.59	16152.13 ± 4780.13	21157.69 ± 9000.30	0.160
Hb (g/dL)	13.17 ± 3.12	12.07 ± 2.24	11.25 ± 2.08	0.407
PCV (%)	32.17 ± 5.17	32.15 ± 6.48	30.15 ± 5.74	0.658
TPP (g/dL)	8.56 ± 1.08	8.40 ± 1.13	8.66 ± 1.36	0.468
Fibrinogen (mg/dL)	208.33 ± 105.22	173.33 ± 83.67	184.62 ± 80.06	0.201

Note: [abc] Means in a row without a common superscript letter differ significantly ($P < 0.05$)

Table 3. Resistance test of gastrointestinal nematodes in pet cats in Yogyakarta against pyrantel pamoate and ivermectin

Group	EPG D0	EPG D10	iFECRT (%)	cFECRT (%)	Status
OP, treated with pyrantel at a dose of 5 mg/kg BW (n= 30)	2031.58 ± 3367.21	15.79 ± 47.30	99.54 ± 1.24	98.48	S
SCI, treated with ivermectin at a dose of 200 µg/kg BW (n=32)	1016.67 ± 1114.15	4.76 ± 15.04	99.75 ± 0.96	99.57	S
C, untreated control (n=36)	970.83 ± 992.50	1037.50 ± 883.29	-19.91 ± 39.63		

iFECRT: Individual fecal egg count reduction test (without untreated control), cFECRT: Control fecal egg count reduction test (with untreated control), S: susceptible

Based on the criteria suggested by Ntampaka et al. (2021) that EPG>550, 150-500, and 50-100 indicate severe, moderate, and mild infections, respectively, so the nematodiasis in this recent study could be categorized as severe. However, the animals were apparently healthy or showing clinical signs of diarrhea, dull hair, anorexia, and vomiting. This is in line with the findings of several previous researchers. Dybing et al. (2018) and Ursache et al. (2021) stated that nematodiasis or helminthiasis is often not accompanied by any clinical symptoms. The animals had an overall body condition score range of 4.56-5.57, which could be categorized as ideal for cats (Bjornvad et al. 2011) and the average value of some hematological and blood chemical parameters, which were still in the physiological range (Ferriani et al. 2022). In contrast to this condition, *Toxocara* and *Ancylostoma* infestation might result in some clinical findings, e.g., diarrhea, dull hair, anorexia, vomiting (Calista et al. 2019; Ursache et al. 2021), anemia, enteritis, and hypoalbuminemia (Epe 2009).

Regardless of the health condition of the animal, the critical thing to note from the recent study is that *Toxocara* and *Ancylostoma* have zoonotic potential (Studzińska et al. 2017; Chen et al. 2018; Castro et al. 2019; Traub et al. 2021; von Samson-Himmelstjerna et al. 2021) and their eggs could contaminate soil and pose a threat to public health. Especially an animal with toxocariasis or ancylostomiasis but showing no clinical symptoms is a severe zoonotic threat. Taking the findings into account that animals are reared and defecated indoors and accessible outside the house (Table 1), the possibility of these infectious agents causing environmental pollution is very high. Environmental studies in several countries, e.g., Poland, Mexico, Brazil and America, showed that *Toxocara* and hookworms were the most prevalent nematode identified in the soil of parks, playgrounds, sports fields, trees and green areas, school and campus ground throughout the year (Bowman et al. 2010; Bojar and Kłapeć 2018; Ferraz et al. 2019; Nava et al. 2020; Stafford et al. 2020; Delai et al. 2021; de Mello et al. 2022). The constant presence of *Toxocara* and *Ancylostoma* eggs in the soil will become a source of infection for dogs, cats, and even humans (von Samson-Himmelstjerna et al. 2021). Moreover, contamination can also occur in infected animal hair. The results of a study on dogs in Brazil showed that *Toxocara* infective eggs were found on the hair around the lower back and anus/perianal area (da Cunha et al. 2010; Delai et al. 2021). Furthermore, it is important to note that the eggs of this nematode have a solid resistance to drought, allowing infection to occur throughout the year (Bojar and Kłapeć 2018; Araújo et al.

2021). In addition, keeping animals by freeing animals to roam outside the house also opens up opportunities for worm transmission through paratenic hosts such as rodents and insects (Matsusaki 1951; Little 1961; Lee et al. 2014).

Based on the findings of this study that roundworm and hookworm infestations in pet cats are zoonotic and found in young and adult cats kept indoors or free-roaming in the environment, attention to treatment and control of nematodiasis must be paid. One of the crucial steps to be able to treat helminthiasis is the proper use of drugs. The results of the iFECRT and cFECRT indicate that the gastrointestinal worms in pet cats in Yogyakarta are still susceptible to pyrantel pamoate and ivermectin. According to Coles et al. (2006), if the FECRT in the anthelmintic resistance test is > 95% and the lower 95% confidence limit is higher than 90, then it can be interpreted that both nematodes are susceptible to both pyrantel pamoate and ivermectin. Furthermore, the FECRT values were more than 98%, so it could be claimed that both anthelmintics were highly effective against both gastrointestinal nematodes (Zekarias and Toka 2019). Therefore, administering pyrantel, ivermectin, or a combination of these drugs will be a choice to treat ancylostomiasis and toxocariasis and to reduce environmental contamination with hookworm and roundworm eggs from owned/pet cats in Yogyakarta.

In conclusion, *Ancylostoma* and *Toxocara* infestations were found in pet cats kept indoors and roamed freely around the house in Yogyakarta. These nematodes were still highly susceptible to the commonly and widely used, namely pyrantel pamoate and ivermectin. Considering these parasitic diseases are zoonotic, it is necessary to take strategic actions to eradicate and control hookworm and roundworm infestations through medical treatment and good animal and environmental management practices.

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