The PHILIPPINE JOURNAL of
Veterinary Medicine

Volume 61 No. 2 July - December 2024

Published by the College of Veterinary Medicine
University of the Philippines Los Baños

The Philippine Journal of Veterinary Medicine

Volume 61 No. 2 July - December 2024

The Philippine Journal of Veterinary Medicine (ISSN 0031-7705 print; eISSN 2984-763X online) is a peer- reviewed international journal of basic, applied, and translational research in veterinary medicine and biomedical science. It is published semi-annually, for the periods January-June and July-December each year, by the College of Veterinary Medicine, University of the Philippines Los Baños. All articles are subjected to double-blind review. Authors of articles appearing in the journal are solely responsible for opinions expressed therein.

All communications should be addressed to:

The Editor-in-Chief
Philippine Journal of Veterinary Medicine
College of Veterinary Medicine
University of the Philippines Los Baños
Laguna, Philippines 4031
Telefax No. +63-49-536-2727
Email: pjvm1964@gmail.com, pjvm.uplb@up.edu.ph

This journal is abstracted/indexed by: SCOPUS, Biological Abstracts, Focus on: Veterinary Science & Medicine, Web of Science Zoological Records, CAB Abstracts, Index Veterinarius, Veterinary Bulletin, Parasitology Database, Helminthological Abstracts, Protozoological Abstracts. Review of Medical and Veterinary Entomology, EBSCO, ASEAN Citation Index, Prescopus Russia, i-journal (www.ijournals.my), i-focus (www.ifocus.my), i-future (www.ifocus.my), Philippine E-Journals (https://ejournals.ph) and UPLB Journals Online (http://journals.uplb.edu.ph/index.php/PJVM).

Scan Me
PJVM latest articles

Scan Me
PJVM Guidelines for Authors

Scan Me
PJVM Form for Authors

Scan Me
PJVM Form for Reviews

Table of Contents

(Original Article)

Companion Animals as Reservoirs and Sentinels for Zoonotic Helminths in Selected Rural Communities in Agusan del Sur and Surigao del Norte, Philippines

Allen Jethro I. Alonte[1,], Sheina Macy P. Manalo[2], Martha E. Betson[3], Billy P. Divina[2], and Vachel Gay V. Paller[1]*

[1] Animal Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna, 4031, Philippines;

[2] Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, Laguna, 4031, Philippines

[3] School of Veterinary Medicine, University of Surrey, Guildford, Surrey, GU2, United Kingdom.

*Correspondence: aialonte@up.edu.ph (Allen Jethro I. Alonte)

Submitted: 29 May 2024 Revised: 10 Jul 2024 Accepted: 04 Nov 2024 Published: 05 Dec 2024

Abstract

Background: The growing population of companion animals and their close interaction with humans contributes to zoonotic transmission and persisting endemicity of intestinal helminths in poor and developing countries, such as the Philippines. This research focused on the potential role of dogs and cats as reservoirs and sentinels of intestinal helminths in selected rural communities in Agusan del Sur and Surigao del Norte, Philippines. Methods: A total of 135 dog and 33 cat stool samples from 120 households were collected and processed using the simple sedimentation and modified McMaster techniques as well as multiplex real-time polymerase chain reaction (RT-PCR). Results: Results showed high intestinal helminth prevalence in dogs and cats from selected communities of Agusan del Sur and Surigao del Norte ranging from 59.7% to 75.0% with hookworm being the most prevalent. Furthermore, results of the multiplex RT-PCR showed possible cross transmission of parasites to non-native host. Conclusions: The findings of the study showed the importance of dogs and cats as reservoirs and sentinels for a wide range of intestinal parasites, suggesting their potential role in the zoonotic transmission of intestinal parasites. It highlights the need for veterinary public health measures in the country to address the gaps in intestinal helminth control.

Keywords: Intestinal helminths, zoonoses, dogs, cats, companion animals

1. Introduction

The close relationship of companion animals, particularly dogs and cats, provides intangible benefits such as emotional and social support to their owners [1,2]. However, they are also known to harbor diverse zoonotic pathogens including parasites. Dogs and cats are associated with more than 60 species of parasites, potentially risking human health and among these are intestinal helminths [3].

Intestinal helminths are among the leading causes of infection in humans, affecting approximately 1.5 billion people worldwide, particularly in low-and middle-income countries [4]. Endemicity persists in impoverished areas where access to safe water and basic sanitation and hygiene (WASH) facilities are insufficient [5]. Intestinal helminthiasis is associated with morbidities such as poor physical and mental development among children and decreased productivity among adults causing economic losses, aggravating existing poverty [6,7]. The vicious cycle of parasitism and poverty makes it a serious public health issue and at the same time an economic burden in poor and developing countries, such as the Philippines [6, 8, 9].

There is increasing evidence that dogs and cats can act as reservoirs of intestinal helminth species that can infect humans, most notably hookworm and *Toxocara* spp. [10]. The increasing global population of dogs and cats [11] as well as the fact that they are free roaming in many settings and their natural propensity to defecate in the open, may mean that they can contribute significantly to environmental contamination with intestinal helminth eggs [12, 13]. Further, the strong bond between companion animals and humans initiates a close physical interaction increasing vulnerability of humans to zoonotic parasites [14,15]. Thus, the World Health Organization (WHO) recommends the implementation of complementary veterinary public health measures in addition to the existing intestinal helminth control and elimination strategies [7]. In the Philippines, however, policies and guidelines supporting this recommendation are yet to be formulated.

It is imperative to have sufficient data prior to the formulation of these policies and guidelines but at present there is lack of data on the prevalence of intestinal helminths in animals hindering successful control. Sentinel surveillance for intestinal helminths in the Philippines is currently focused on humans [16], however, the evident zoonotic transmission as well as risky behavior of companion animals calls the need for their inclusion in sentinel surveillance [10, 7]. As such, this research aims to assess the potential role of companion animals as reservoir hosts and sentinel species of intestinal helminths in selected rural communities in Agusan del Sur and Surigao del Norte. To our knowledge, this paper is the first to look into the potential role of dogs and cats as reservoirs and sentinel population for intestinal helminthiasis in the region, practically serving as baseline data providing a foundation for research-based policy recommendations for responsible animal ownership and integrated control and elimination of parasitic diseases in endemic rural communities.

2. Materials and Methods

Study design and setting

This cross-sectional study was conducted as part of the Zoonotic Transmission of Intestinal Parasites (ZooTRIP project) [17] in selected rural communities in the provinces of Agusan del Sur and Surigao del Norte, the Philippines. Two rural communities from each province were selected based on known endemicity of intestinal helminths, willingness of local government units (LGUs) to cooperate, accessibility of communities, and the security situation. Recruitment to the ZooTRIP project [17] was done at household level. Households were randomly selected from the list of the village and only households which had dog/s and/or cat/s were included in this study.

Sample and data collection

Household visits were conducted by the study team to collect samples. Freshly voided stool samples (approximately 10g) were collected from dogs and cats present in the household by picking up stools using a spatula from the usual spots where these animals defecate. In cases where stools were unavailable at the time of household visit, the owners were given specimen cups and spatula and were advised to collect freshly excreted stool samples from each animal and submit to the village center the following day. Owners were also interviewed to gather information such as sex and age, and breed of companion animals as well as socio-demographic profile and animal care practices of households. Upon receipt at the village center, stool samples were preserved using 95% ethanol and immediately transported in a cold chain to the laboratory for immediate processing and examination.

Sample processing and examination

Collected stool samples were processed using simple sedimentation and modified McMaster techniques. In summary, the simple sedimentation technique was performed by mixing 1 g of stool sample with 9 mL distilled water using a vortex mixer (Vortex-Genie®, Scientific Industries, USA). The mixture was sieved using a 125 µm wire mesh followed by centrifugation for 10 minutes. The supernatant was decanted, and duplicate smears were prepared from the sediment for immediate microscopic examination [18]. For the modified McMaster technique, a stool quantitative analysis kit (Paracoun-EPG™, U.S.A.) with the accompanying instructions was used with minor modifications. Briefly, 4 g of stool sample and 26 mL of Sheather's solution (sucrose + 10% formalin solution) were thoroughly mixed in the calibrated mixing vial. The mixture was filtered through a 1-mm metal sieve and the filtrate was again placed in the calibrated mixing vial. Sheather's solution was added to reconstitute the 30 mL volume of the mixture followed by thorough mixing. The mixture was then loaded to the two loading chambers of the McMaster slide and was allowed to sit for at least five minutes to allow flotation to occur [18] before microscopic examination. All examinations were performed by trained microscopists. The number of helminth eggs per parasite were counted and eggs per gram feces (epg) were determined. Identification of helminth eggs was based on the morphologic features and size as described by Zajac and Conboy [18].

Molecular identification of select zoonotic helminths

Multiplex real-time PCR assays were used to detect the presence of select zoonotic helminths including *Ancylostoma* spp., *Necator americanus*, *Toxocara cati*, *T. canis*, and *Schistosoma japonicum.* Due to limitations in resources, only these common zoonotic helminths were considered for detection. DNA was extracted from 600 µL stool-ethanol suspension using DNeasy Powersoil Kit (Qiagen, USA). Multiplex assay 1 (MP1) was used to amplify and detect the ITS1 region of *N. americanus* and *Ancylostoma* spp. following the protocol of [19]. For the detection of *S. japonicum*, NADH1 gene was used following Lier *et al.*, [20]. On the other hand, MP3 was used to amplify and detect the ITS2 of *T. canis* and *T. cati* following Durant *et al.*, [21]. PCR assays were run in 10 µL reaction volumes containing 5 µL mastermix (QuantiTect Multiplex PCR NoROX Kit, Qiagen), 10 µM of each primer and probe, 2 µL DNA template, and was adjusted to final volume using nuclease-free water. Primer sets and probes are shown in Table 1.

Assays were performed using the Magnetic Induction Cycler (MIC) qPCR cycler (Bio Molecular Systems, Australia). The optimized amplification profiles are set for 15 min at 95°C initialization, followed by 40 cycles (MP1 and MP2)/45 cycles (MP3) of denaturation for 60 secs at 94°C, and 60 secs at 60°C (MP1 and MP3)/55°C(MP1). Upon optimization of the protocol using known amount of DNA (based on the number of eggs) for each parasite, a Cq value of ≤35 was used as a cut-off to distinguish between positive and negative samples. A positive and

Table 1. List of primers and probes used for the detection of select zoonotic helminths in dogs and cats in selected rural communities of Agusan del Sur and Surigao del Norte, the Philippines

Assay	Target organism	Primer/Probe	Primer/Probe sequence	Target bp	Reference
MP1	*Necator americanus*	Fwd	5'-CTGTTTGTCGAACGGTACTTGC-3'	101	Verweij et al., (2007)
		Rev	5'-ATAACAGCGTGCACATGTTGC-3'		
		Probe	5'-[6FAM]CTGTACTACGCATTGTATAC[MGBNFQ]-3'		
	Ancylostoma spp.	Fwd	5'-GAATGACAGCAAACTCGTTGTTG-3'	71	Verweij et al., (2007)
		Rev	5'-ATACTAGCCACTGCCGAAACGT-3'		
		Probe	5'-[VIC] ATCGTTTACCGACTTTAG [MGBNFQ]-3'		
MP2	*Schistosoma japonicum*	Fwd	5'-ACTGGTTATGGTTTGTTGATGTTAGGT-3'	88	Wiria et al., (2010)
		Rev	5'-AGCCACACGAACAGCACTAATC-3'		
		Probe	5'-[VIC] AGGTTCTTGGAAAAAGTAT [MGBNFQ]-3'		
MP3	*Toxocara canis*	Fwd	5' -ACGCGTACGTATGGAATGTGCT-3'	141	Durant et al., (2012)
		Rev	5' -GAGCAAACGACAGCSATTTCTT-3'		
		Probe	5'-[HEX]TCTTTCGCAACGTGCATTCGGTGA[MGB-Eclipse®]-3'		
	Toxocara cati	Fwd	5' -GCGCCAATTTATGGAATGTGAT-3'	155	Durant et al., (2012)
		Rev	5' -GAGCAAACGACAGCSATTTCTT-3'		
		Probe	5'-[FAM]CCATTACCACACCAGCATAGCTCACCGA[BHQ1]-3'		

negative control were included in each run. Lastly, internal amplification control (PrimerDesign, UK) was added in each run to check possible PCR inhibitions

Ethical considerations

Ethical approval for the ZooTRIP project was obtained from the University of Philippines Manila Research Ethics Board (UPMREB 2019-084-01), the Animal Care and Use Committee of University of Philippines Los Baños (CAS-2018-020) and the University Ethics Committee of University of Surrey (UEC 2019 049). In addition, the study was reviewed by the Animal Welfare and Ethical Review Board of the University of Surrey (OUT036) and found to conform to the expectations of the University, regarding ethics and good practice of animal experimentation. Prior to project implementation, the study team consulted and obtained permission from the Department of Agriculture (DA) and concerned LGUs. Informed consent of owners was obtained before the collection of stool samples. All personal information identifying animal owners was replaced with a unique identifier to ensure anonymity. Only members of the study team had access to the data to maintain privacy and confidentiality strictly following the Data Privacy Act of 2012. Feedback meetings were conducted to report the results of the parasitologic assessment to DA and concerned local government offices for appropriate treatment and management.

Data processing and analysis

The gathered data were encoded in Microsoft Excel 2010 and were rechecked to ensure accuracy of encoding. All discrepancies observed were resolved as necessary by rechecking the original document.

Prevalence and intensity of detected parasites were determined. The Pearson chi-squared test was used to determine significant differences in prevalence relative to sex, age group, and breed. Age group was defined as 0-12 months and greater than 12 months old. Multiple logistic regression analyses were also used to identify risk factors associated with intestinal helminthiases. Intestinal helminth infection status was used as outcome to determine shared risk factors of being infected with intestinal helminths. Bivariate analysis of outcome and variables was carried out prior to the regression analysis. Significant variables were used in the regression model. Backward stepwise multiple logistic regression analysis was done to identify predictive variables in the final model. In all analyses, a p-value of less than 0.05 was considered significant. Data management and analysis were performed using STATA 17 (STATA 17®, Stata Corp, Texas, USA).

3. Results

A total of 135 dog and 33 cat stool samples were collected from 120 households at the study sites. Results revealed an overall intestinal helminth prevalence of 75.0% (95% CI: 63.6-83.8) and 59.7% (95% CI: 47.7-70.6) in dogs of Agusan del Sur and Surigao del Norte, respectively. Ten helminth species were seen including nematodes (hookworms, *Toxocara* spp., *Trichuris* spp., *Gnathostoma* spp., and *Capillaria hepatica*), trematodes (*Schistosoma japonicum*, heterophyids, and *Fasciolopsis buski*), and cestodes (*Spirometra* spp., and *Dipylidium caninum*). The predominant parasite observed in dogs was hookworm, detected in 57.3% and 50.7% of the examined samples in Agusan del Sur and Surigao del Norte, respectively. Out of all the intestinal helminths recorded, *Gnathostoma* spp., *Fasciolopsis buski*, and *Dipylidium caninum* were only detected in Agusan del Sur (Table 2). In Agusan del Sur, the highest mean intensity was seen in *Toxocara* spp. with 488 epg, similar to Surigao del Norte 546 epg. Mean intensity of other intestinal helminths is detailed in Table 2.

Table 3 summarizes the prevalence and mean intensity of intestinal helminths observed in cats. An overall intestinal helminth prevalence of 66.7% was observed in cats of Agusan del Sur and Surigao del Norte. Six helminth species were observed in cats (hookworm, *Toxocara* spp., *Trichuris* spp., *S. japonicum*, *Spirometra* spp., and *Platynosomum* spp.). Hookworm is the most prevalent helminth seen in Agusan del Sur with a prevalence of 61.1%, while *Toxocara* spp. was the most prevalent in Surigao del Norte with a prevalence of 33.3%. Two of the intestinal

Table 2. Prevalence and mean intensity of intestinal helminths in dogs in selected rural communities of Agusan del Sur (N=68) and Surigao del Norte (N=67), the Philippines

Intestinal helminths	Agusan del Sur			Surigao del Norte		
	No. of positive n (%)	95% CI[a]	Mean Intensity eggs/g (SD[b])	No. of positive n (%)	95% CI[a]	Mean Intensity eggs/g (SD[b])
Dogs						
Hookworm	39 (57.3)	45.5-68.4	124.7 (283.9)	34 (50.7)	39.1-62.3	240.5 (621.9)
Toxocara spp.	16 (23.5)	15.0-34.9	488.3 (731.9)	8 (11.9)	6.2-21.8	546.2 (922.8)
Trichuris spp.	7 (10.3)	5.1-19.8	40.6 (25.7)	4 (6.0)	2.3-14.4	40.5 (14.5)
Schistosoma japonicum	1 (1.5)	0.3-7.9	36.0 -	2 (3.0)	0.8-10.2	14 -
Spirometra spp.	8 (11.8)	6.1-21.5	291.8 (381.8)	1 (1.5)	0.3-8.0	2 -
Heterophyids	1 (1.5)	0.3-7.9	19.0 -	1 (1.5)	0.3-8.0	19 -
Gnathostoma spp.	1 (1.5)	0.3-7.9	18.0 -	0 -	-	- -
Fasciolopsis buski	1 (1.5)	0.3-7.9	15.0 -	0 -	-	- -
Capillaria hepatica	1 (1.5)	0.3-7.9	25.0 -	1 (1.5)	0.3-8.0	2 -
Dipylidium caninum	1 (1.5)	0.3-7.9	25.0 -	0		-
Any parasite	51 (75.0)	63.6-83.8	- -	40 (59.7)	47.7-70.6	- -

[a] 95% Confidence Interval
[b] Standard Deviation
[c] eggs and larvae/g

Table 3. Prevalence and mean intensity of intestinal helminths in cats in selected rural communities of Agusan del Sur (N=18) and Surigao del Norte (N=15), the Philippines

Parasite	Agusan del Sur			Surigao del Norte		
	No. of positive n (%)	95% CI[a]	Mean Intensity eggs/g (SD[b])	No. of positive n (%)	95% CI[a]	Mean Intensity eggs/g (SD[b])
Cats						
Hookworm	11 (61.1)	38.6-79.7	29.0[c] (25.0)	3 (20.0)	7.0-45.2	664 (877.7)
Toxocara spp.	6 (33.3)	16.3-56.2	251 (274.9)	5 (33.3)	15.2-58.3	338 (668.5)
Trichuris spp.	2 (11.1)	3.1-32.8	23 (2.8)	1 (6.7)	1.2-29.8	31 -
S. japonicum	1 (5.6)	1.0-25.8	25 -	0 -	-	- -
Spirometra spp.	1 (5.6)	1.0-25.8	26 -	1 (6.7)	1.2-29.8	3,625
Platynosomum spp.	2 (11.1)	3.1-32.8	39 (19.1)	0 -	-	- -
Any parasite	12 (66.7)	43.7-83.7	- -	10 (66.7)	41.7-84.8	- -

[a] 95% Confidence Interval
[b] Standard Deviation
[c] eggs and larvae/g

helminths, namely *S. japonicum* and *Platynosomum* spp., were only present in the samples examined from Agusan del Sur. Among the intestinal helminths of cats, *Toxocara* spp. has the highest mean intensity with 251 epg in Agusan del Sur, while in Surigao del Norte *Spirometra* spp. has the highest with 3,625 epg.

Multiple infection with intestinal helminths was also observed in dogs and cats at the study sites. The majority of the infected dogs and cats only harbored one parasite (45.9% and 42.4%, respectively). Double and triple infection of intestinal parasites was also observed in the study sites with prevalence rates reaching up to 16.3% for double infection and 9.1% for triple infection (Table 4). The dogs and cats were also grouped and analyzed according to age and sex. Grouping them according to breed was not attempted as almost all companion animals included in the study have mixed breeds. *Trichuris* spp. infection was only seen in male cats resulting to significantly higher prevalence than female cats ($p=0.034$) (Table 5). Overall, results showed no significant difference in the prevalence of intestinal helminths when grouped as to age and sex of dogs and cats ($p>0.05$) (Table 5). Disaggregated prevalence as to age and sex can be found in Supplementary Table 1.

Table 6 summarizes the animal care practices in the households surveyed. Higher mean population of cats (1.4, between 0-11) than dogs (0.7, between 0-5) in each household was observed. Most of the dogs and cats in the study sites were freely roaming (55.8%). The majority of the households in Agusan del Sur revealed that they have cleaned their pets' waste every day (46.3%) compared to Surigao del Norte where most households never practiced cleaning up after their animals (63.3%). Overall, 48.3% of the households

Table 4. Prevalence of single and multiple infection of intestinal helminths in dogs (N=135) and cats (N=33) in selected rural communities of Agusan del Sur and Surigao del Norte, the Philippines

Parasitic infection	Dogs		Cats		Total	
	Prevalence n (%)	95% CI[a]	Prevalence n (%)	95% CI[a]	Prevalence n (%)	95% CI[a]
Single infection	62 (45.9)	37.7-54.3	14 (42.4)	27.2-59.2	76 (45.2)	37.9-52.8
Double infection	22 (16.3)	11.0-23.4	5 (15.2)	6.6-30.9	27 (16.1)	11.3-22.4
Triple infection	7 (5.2)	2.5-10.3	3 (9.1)	3.1-23.6	10 (6.0)	3.3-10.6

[a] *95% Confidence Interval*

Table 5. Comparison of prevalence (p-value) of select intestinal helminths relative to age group and sex of dogs and cats in selected rural communities of Agusan del Sur and Surigao del Norte, the Philippines

Parasite	Dogs		Cats	
	Age Group	Sex	Age Group	Sex
Hookworm	0.394	0.051	0.566	0.653
Toxocara spp.	0.109	0.682	1.000	0.213
Trichuris spp.	0.414	0.110	0.439	0.034*
Schistosoma japonicum	0.516	0.082	0.354	0.237
Any parasite	0.161	0.076	1.000	0.803

* *significant at 5% level of significance*

Table 6. Practices related to animal care of households surveyed in Agusan del Sur (N=54) and Surigao del Norte (N=66), the Philippines

Characteristics	Agusan del Sur n (%)	Surigao del Norte n (%)	Total n (%)
Mean number of dogs in the household (range)	0.7 (0-4)	0.7 (0-5)	0.7 (0-5)
Mean number of cats in the household (range)	1.8 (0-11)	1.1 (0-4)	1.4 (0-11)
Mobility of pets			
Inside the house	1 (1.8)	8 (12.1)	9 (7.5)
Confined in a cage	10 (18.5)	22 (33.3)	32 (26.7)
Leashed	12 (22.2)	0	12 (10.0)
Freely roaming	31 (57.4)	36 (54.6)	67 (55.8)
Frequency of cleaning pets' waste			
Everyday	25 (46.3)	16 (24.2)	41 (34.2)
At least once a week	13 (24.1)	8 (12.1)	21 (17.5)
Never	16 (29.6)	42 (63.6)	58 (48.3)
Pet's waste disposal			
Leave on the ground	12 (22.2)	36 (54.5)	48 (40.0)
Throw in the trash can	4 (7.4)	5 (7.6)	9 (7.5)
Flush down the toilet	2 (3.7)	0	2 (1.7)
Bury	36 (66.7)	25 (37.9)	61 (50.8)
Check-up with veterinarian			
Yes	17 (31.5)	13 (19.7)	30 (25.0)
No	37 (68.5)	53 (80.3)	90 (75.0)
Deworming of pets	13 (24.1)	13 (19.7)	26 (21.7)
Yes	19 (35.2)	10 (15.2)	29 (24.2)
No	35 (64.8)	56 (84.8)	91 (75.8)
No. of households with at least one infected pet	43 (79.6)	43 (65.1)	86 (71.7)

surveyed never cleaned their animals' waste, 34.2% cleaned every day, while 17.5% practiced cleaning-up at least once a week. Owners in both provinces disposed animal waste mostly through burying (50.8%), followed by leaving waste on the ground (40%), throwing it in the trash can (7.5%), and very few flush it down the toilet (1.7%). Seventy-five percent (75%) of the households surveyed do not visit veterinary clinics for check-up, hence, more households (75.8%) do not have their animals dewormed. Of the 120 households, 86 (71.7%) households were found to own at least one companion animal with intestinal helminthiasis.

The logistic regression model showed no significant animal care practices affecting companion animal infection of intestinal helminths (Supplementary Table 2).

Furthermore, Table 7 shows the prevalence of select zoonotic helminths in dogs and cats using real-time PCR. In dogs, *S. japonicum* has the highest prevalence at 92% (95% CI: 86.9-97.0), followed by *Ancylostoma* spp. with 90.2% (95%

Table 7. Molecular prevalence of select zoonotic helminths in dogs and cats in selected rural communities of Agusan del Sur and Surigao del Norte, the Philippines

Intestinal helminths	Agusan del Sur		Surigao del Norte		Overall	
	No. of positive n (%)	95% CI[a]	No. of positive n (%)	95% CI[a]	No. of positive n (%)	95% CI[a]
Dogs						
S. japonicum	53 (89.8)	82.1-97.5	50 (94.3)	88.1-100.0	103 (92.0)	86.9-97.0
N. americanus	8 (13.6)	4.8-22.3	0 -	-	8 (7.1)	2.4-11.9
Ancylostoma spp.	58 (98.3)	95.0-100.0	43 (81.1)	70.6-91.7	101 (90.2)	84.7-95.7
T. canis	8 (13.6)	4.8-22.3	4 (7.5)	0.4-14.7	12 (10.7)	5.0-16.4
T. cati	0 -	-	1 (1.9)	0.0-5.5	1 (0.9)	0.0-2.6
Cats						
S. japonicum	8 (66.7)	40.0-93.3	9 (81.8)	59.0-100.0	17 (73.9)	56.0-91.9
N. americanus	0 -	-	0 -	-	0 -	-
Ancylostoma spp.	9 (75.0)	50.5-99.5	10 (90.9)	73.9-100.0	19 (82.6)	67.1-98.1
T. canis	1 (8.3)	0.0-24.0	0 -	-	1 (4.3)	0.0-12.7
T. cati	2 (16.7)	0.0-37.7	4 (36.4)	7.9-64.8	6 (26.1)	8.1-44.0

[a] *95% Confidence Interval*

CI:84.7-95.7), *T. canis* (10.7%; 95% CI: 5.0-16.4), *N. americanus* (7.1%; 95% CI: 2.4-11.9), and *T. cati* (0.9%; 95% CI: 0.0-2.6). In cats, on the other hand, highest prevalence was seen in *Ancylostoma* spp. with 82.6% (95% CI: 67.1-98.1), followed by *S. japonicum* with 73.9% (95%CI: 56.0-91.9), N. americanus (34.8%; 95% CI: 15.3-54.2), T. cati (26.1%; 95% CI: 8.1-44.0), and *T. canis* (4.3%; 95% CI: 0.0-12.7).

4. Discussion

The increasing population of dogs and cats in the Philippines poses a serious public health risk for they are considered as major reservoir hosts of several pathogens, including intestinal helminths [10, 22]. Zoonotic transmission of these pathogens may cause emergence or re-emergence of several infectious diseases. Thus, the WHO recommends implementation of veterinary public health measures in the control and elimination of infectious diseases, such as intestinal helminthiasis [7]. However, there is a significant lack of guidance by way of policy in the Philippines to implement the said measures.

The results of the study showed high prevalence of diverse helminth species and evidence of multiple infection among companion animals in the selected study sites, confirming that they are important reservoir hosts of intestinal helminths. Among these, hookworms were found to have the highest prevalence with rates reaching up to 70.6%. The only other study in the Philippines investigating hookworm in companion animals from Northern Samar detected hookworm eggs in 33% (29/87) of dog stool samples by Kato Katz technique [23]. Hookworm infections have been detected in dog and cat populations in other countries in southeast Asia with prevalence ranging from 13.9% to 89.5%, depending on host species and location [24-34]. Based on a systematic review, the prevalence of hookworm infection in humans in the Philippines has been estimated at 26% [35], although there is significant heterogeneity in prevalence between regions [36]. Moreover, in this present study, the human hookworm, N. americanus, as it has been rarely detected in other mammalian species [37,38], was detected among dogs using multiplex real-time PCR. Similar to the previous findings [39], N. americanus was detected among dogs and pigs implying possible role of these animals in the zoonotic transmission of the hookworms. However, the possibility of it being a spurious parasite of dogs and pigs cannot be discounted and should be investigated further. A recent study has reported evidence of Ancylostoma ceylanicum in dogs and humans in the municipalities of Palapag and Laoang, Northern Samar, Philippines [23], providing further evidence of the potential for zoonotic transmission. Several molecular analyses also revealed the presence of hookworm species with zoonotic potential in both human and domestic animals [24-30, 34].

Another zoonotic parasite found to have high prevalence was *Toxocara* spp. with rates reaching up to 33.3%. The result of this study is also consistent with several epidemiological studies on companion animals with rates ranging from 4.2% - 48% in cats and dogs [26, 40-43]. It is unusual to detect these parasites in humans using diagnostic stool microscopy since they are not capable of harboring adult worms. As such, toxocariasis was not yet reported in any sentinel surveillance for intestinal parasites in the Philippines which are annually conducted using Kato-Katz technique [16,44]. Toxocariasis causes significant morbidity and loss of productivity in children, thus posing a serious public health problem, yet it is still neglected [45]. The close interaction of humans, especially children, with companion animals increases the risk of zoonotic transmission. An alarmingly high seroprevalence of *Toxocara* spp. reaching up to 49.0% in children was previously observed in a rural community in the Philippines indicating exposure to the parasite [46, 47]. The often-overlooked presence of *Toxocara* spp. may increase the risk of transmission to humans, possibly risking significant morbidity [45]. Inclusion of animals in sentinel surveillance will help in identifying high transmission areas not only for *Toxocara* spp. but also, for other zoonotic parasites.

Significantly higher *Trichuris* spp. prevalence in male cats compared to female cats was observed in the study. This is similar to the findings of Krone *et al.*, [48] but in contrast to the findings of several researches [49-52]. This may be due to sex-specific differences such as hormonal differences in wild cats resulting into a sex-biased parasitism [48], although further studies are required to prove this biased parasitism. On the other hand, results showed that the prevalence of intestinal helminths in companion animals is not significantly different between age groups and sexes similar to the findings of Awoke *et al.* [53] and Szwabe and Blaszkowska [11]. In contrast, several researches [51, 54-56] found higher prevalence in younger dogs (below 12 months) than older dogs. These findings may imply that companion animals regardless of age and sex are highly susceptible to intestinal helminthiasis and are capable of harboring and transmitting these parasites.

Findings of the study also suggested low awareness of owners in the potential role of animals in the continuing endemicity of intestinal helminths. This is evident in the animal care practices of the owner. The majority of the owners bury or leave the animal stool waste in the soil contaminating the environment, thus contributing to ongoing transmission. Most of the owners also allow animals to freely roam the ground, increasing the risk of being infected. The study of Galgamuwa *et al.*, [57] showed that stray dogs have a higher risk for infection compared to confined ones. The persistent exposure to reservoirs of parasites and the lack of competent veterinary care, especially anthelmintic medicines, could have contributed to the higher prevalence of parasitic diseases observed among stray dogs [57]. Several studies showed that confinement or caging of companion animals limits its exposure and risk of acquiring parasitic infection of both the companion animals and its owner [58-60]. Confinement not only limits the likelihood of acquiring infection of susceptible companion animals but also reduces soil contamination through the feces of infected animals reducing environmental contamination.

In addition, our results showed inadequate deworming practices and veterinary consultation for companion animals. The related poor socio-economic conditions in the area may have contributed to these findings. Unlike in humans, there are no existing mass deworming programs for animals. Companion animals are usually dewormed after check-up with veterinarians. However, the added cost for check-up of animals or transportation to local veterinary clinics cannot be afforded and is not a priority for most owners in endemic areas.

The practices mentioned contribute to the ongoing transmission and may spread endemicity of intestinal parasites in the area. It was demonstrated in Sri Lanka that increasing knowledge of animal owners leading to responsible animal care has resulted to a steady decrease in the prevalence of parasitic infection such as *Toxocara* spp. over the years [57]. Educating animal owners on the role of companion animals as well as the zoonotic risk of parasitic infections through health education campaigns may lead to activate participation of the owners in the control and prevention of parasitic infections [61].

Results of the multiplex real-time PCR allowed species identification of the zoonotic helminths showing possible cross transmission of parasites to non-native host. As seen in the case of *N. americanus*, where it was detected among dogs although it is widely known that humans are its definitive host. However, due to limitations in the resources only these hookworm species were included for detection. Furthermore, *T. canis*, an ascarid of dog, was also seen in cat; while *T. cati*, an ascarid of cat, was seen in dog. Although these findings provide preliminary evidence of cross transmission, further molecular studies may be required to fully map out the transmission pathways among these hosts. Furthermore, the multiplex real-time PCR provides more sensitive detection of *S. japonicum*. Microscopic detection only shows prevalence rates as high as 5.6%, in contrast with molecular detection showing up to 92.0% prevalence rate. Considering the discrepancy between the two techniques, gross underreporting of cases is likely when the more affordable microscopic technique is used for surveillance. This shows the need for more advanced techniques to further accelerate control and elimination strategies of these zoonotic helminths.

Policies and legislation to provide guidance for responsible animal ownership exist in the Philippines. However, many are unaware of the existence of these, leading to irresponsible ownership [62]. For example, the Animal Welfare Act of 1998 and Anti-Rabies Act of 2007 suggest not letting dogs and cats roam freely, which contrasts with the observed practice of owners at the study sites. Furthermore, policies in the Philippines are rather focused on the control and elimination of rabies in animals [62-63] while the equally important neglected tropical disease – intestinal helminthiasis – is often overlooked. As national control programs for intestinal helminthiasis only focus on humans, the neglected population of companion animals serves as potential sources of infection continuing the transmission of zoonotic helminths such as hookworm and *Toxocara* spp., which contributes to persisting endemicity [10]. To successfully control intestinal parasites, control strategies focused on animals should be integrated in the existing national control programs. The findings of the study highlight the dire need for veterinary public health measures in the country. It is high time that the country also implement strategies to control these zoonotic helminths.

5. Conclusions

The findings of the study showed the importance of dogs and cats as reservoir hosts and sentinel species for a wide range of intestinal helminths in selected communities in the Philippines, suggesting the potential role of these companion animals in the zoonotic transmission of intestinal parasites. The findings also suggested the need to address the gap in controlling intestinal helminthiasis in animals through veterinary public health measures to disrupt zoonotic transmission and environmental contamination of intestinal parasites. Furthermore, health education is necessary to increase the awareness of the public in the contribution of companion animals in the persisting endemicity of intestinal parasites in the country. The status of intestinal helminthiasis in companion animals stresses the importance of strengthened collaboration between the animal health sector, human health sector, and environmental health sector in accelerating the control and elimination of intestinal parasitic infections in the Philippines.

Availability of Data and Materials

The data that support the findings of this study are available upon reasonable request.

Author Contributions

Ethics Approval and Consent to Participate

Ethical approval for the Animal Care and Use Committee of University of and the University Ethics Committee of University of In addition, the study was reviewed by the Animal Welfare and Ethical Review Board of the and found to conform to the expectations of the University, regarding ethics and good practice of animal experimentation.

Acknowledgment

The authors would like to acknowledge partner institutions, namely Department of Agriculture, Department of Health-Center for Health Development Caraga and the local government units of Agusan del Sur and Surigao del Norte, for their invaluable support and contribution for this project. The authors would also like to thank the Department of Science and Technology-Philippine Council for Health Research and Development and the Medical Research Council, United Kingdom for providing funds for the project.

Funding

This work was supported by the Newton Fund awarded through the Department of Science and Technology-Philippine Council for Health Research and Development and the Medical Research Council, United Kingdom

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Mubanga, M., Byberg, L., Nowak, C., Egenvall, A., Magnusson, P. K., Ingelsson, E., & Fall, T. (2017). Dog ownership and the risk of cardiovascular disease and death – A nationwide cohort study. *Scientific Reports, 7*(1), 1–9. https://doi.org/10.1038/s41598-017-16118-6

[2] Tompkins, D. M., Greenman, J. V., Robertson, P. A., & Hudson, P. J. (2000). The role of shared parasites in the exclusion of wildlife hosts: *Heterakis gallinarum* in the ring-necked pheasant and the grey partridge. *Journal of Animal Ecology, 69*(5), 829–840. https://doi.org/ 10.1046/j. 13652656.2000. 00439.

[3] Macpherson C. N. (2005). Human behaviour and the epidemiology of parasitic zoonoses. *International Journal for Parasitology 35*(11-12), 1319–1331. https://doi.org/10.1016/j. ijpara. 2005.06.004

[4] World Health Organization. (2023). *Soil-transmitted helminth infections*. https://www.who. int/news-room/fact sheets/ detail/ soil-transmitted-helminth-infections

[5] Lorenzo, P. J., Manzanilla, D. R., Cortel, D. K., & Tangog, E. (2019). Community perceptions of mass drug administration for soil-transmitted helminthiasis and schistosomiasis in selected schools in the Philippines. *Infectious Diseases of Poverty, 8*(1), 1–10. https://doi.org/ 10.1186/s40249-019-0595-8

[6] Engels, D., & Zhou, X.-N. (2020). Neglected tropical diseases: An effective global response to local poverty-related disease priorities. *Infectious Diseases of Poverty, 9*(1), 10. https://doi.org/10.1186/s40249-020-0630-9

[7] World Health Organization. (2020). *Ending the neglect to attain sustainable development goals - A road map for neglected tropical diseases 2021–2030*. http://apps.who. int/ bookorders

[8] King, C. H. (2010). Parasites and poverty: The case of schistosomiasis. *Acta Tropica, 113*(2), 95–104. https://doi.org/ 10.1016/ j.actatropica .2009. 11.012

[9] Rees, C. A., Hotez, P. J., Monuteaux, M. C., Niescierenko, M., & Bourgeois, F. T. (2019). Neglected tropical diseases in children: An assessment of gaps in research prioritization. *PLOS Neglected Tropical Diseases, 13*(1), e0007111. https://doi.org/ 10.1371/journal. pntd.0007111

[10] Betson, M., Alonte, A., Ancog, R.C., Aquino, A., Belizario, V. Y., Jr, Bordado, A., Clark, J., Corales, M., Dacuma, M. G., Divina, B.P., Dixon, M.A., Gourley, S. A., Jimenez, J., Jones, B.P., Manalo, S., Prada, J.M., van Vliet, A., Whatley, K., & Paller, V. (2020). Zoonotic transmission of intestinal helminths in Southeast Asia: Implications for control and elimination. *Advances in Parasitology, 108*, 47–131. https://doi.org/ 10.1016/bs.apar.2020.01.036

[11] Szwabe, K., & Blaszkowska, J. (2017). Stray dogs and cats as potential sources of soil contamination with zoonotic parasites. *Annals of Agricultural and Environmental Medicine, 24*, 39–43. https://doi.org/10.5604/ 12321966.1234003

[12] Ortega-Pacheco, A., Torres-Acosta, J.F.J., Alzina-López, A., Gutiérrez-Blanco, E., Bolio-González, M.E., Aguilar-Caballero, A.J., Rodríguez-Vivas, R.I., Gutiérrez-Ruiz, E., Acosta-Viana, K.Y., Guzmán-Marín, E., Rosado-Aguilar, A., & Jiménez-Coello, M. (2015). Parasitic zoonoses in humans and their dogs from a rural community of tropical Mexico. *Journal of Tropical Medicine*, 2015. https://doi.org/10.1155/2015/ 481086

[13] Sato, M. O., Adsakwattana, P., Fontanilla, I. K. C., Kobayashi, J., Sato, M., Pongvongsa, T., Fornillos, R. J. C., & Waikagul, J. (2019). Odds, challenges and new approaches in the control of helminthiasis, an Asian study. *Parasite Epidemiology and Control, 4*, e00083. https://doi.org/10.1016/j.parepi. 2018.e00083

[14] Ratschen, E., Shoesmith, E., Shahab, L., Silva, K., Kale, D., Toner, P., Reeve, C., & Mills, D. S. (2020). Human-animal relationships and interactions during the Covid-19 lockdown phase in the UK: Investigating links with mental health and loneliness. *PLOS One, 15*(9 September), 1–17. https://doi.org/ 10.1371 journal.pone.0239397

[15] Chavez, G. C. S., Paller, V. G., & Lorica, R. P. (2021). Zoonotic Enteroparasites of *Macaca fascicularis* in Palawan, Philippines. *Research Square*; 2021. https://doi.org/ 10.21203/rs.3.rs-861042/v1.

[16] Department of Health. (2016). *Department Memorandum Order 2016-0212: Guidelines on the Implementation of Harmonized Schedule and Combined Mass Drug Administration for the Prevention and Control of Lymphatic Filariasis, Schistosomiasis, and Soil-transmitted Helminthiasis.*

[17] Kajero OT, Janoušková E, Bakare EA, Belizario V, Divina B, Alonte AJ, Manalo SM, Paller V.G., Betson M., & Prada J.M. (2022). Co-infection of intestinal helminths in humans and animals in the Philippines. *Transactions of the Royal Society of Tropical Medicine and Hygiene 116*(8):727–735. doi:10.1093/trstmh/trac 002.

[18] Zajac, A. M., & Conboy, G. A. (2011). *Veterinary Clinical Parasitology* (8th ed.). Wiley-Blackwell.

[19] Verweij, J.J., Brienen, E.A., Ziem, J., Yelifari, L., Polderman, A.M., & Van Lieshout, L. (2007). Simultaneous detection and quantification of *Ancylostoma duodenale, Necator americanus,* and *Oesophagostomum bifurcum* in fecal samples using multiplex real-time PCR. *The American Journal of Tropical Medicine and Hygiene, 77*(4), 685–690.

[20] Lier, T., Simonsen, G.S., Wang, T., Lu, D., Haukland, H.H., Vennervald, B.J., Hegstad, J., & Johansen, M.V. (2009). Real-time polymerase chain reaction for detection of low-intensity *Schistosoma japonicum* infections in China. *The American Journal of Tropical Medicine and Hygiene, 81*(3), 428–432.

[21] Durant, J.F., Irenge, L.M., Fogt-Wyrwas, R., Dumont, C., Doucet, J.P., Mignon, B., Losson, B., & Gala, J.L. (2012). Duplex quantitative real-time PCR assay for the detection and discrimination of the eggs of *Toxocara canis* and *Toxocara cati* (Nematoda, Ascaridoidea) in soil and fecal samples. *Parasites & Vectors, 5*, 288. https://doi.org/10.1186/1756-3305-5-288

[22] Nguyen, V. L., Dantas-Torres, F., & Otranto, D. (2021). Canine and feline vector-borne diseases of zoonotic concern in Southeast Asia. *Current Research in Parasitology & Vector-Borne Diseases, 1*(November 2020), 100001. https://doi.org/10.1016/j. crpvbd. 2020.100001

[23] Aula, O.P., McManus, D.P., Weerakoon, K.G., Olveda, R., Ross, A.G., Rogers, M.J., &

Gordon, C.A. (2020). Molecular identification of *Ancylostoma ceylanicum* in the Philippines. *Parasitology, 147*(14), 1718–1722. https://doi.org/10.1017/ S0031182020001547

[24] Conlan, J.V., Khamlome, B., Vongxay, K., Elliot, A., Pallant, L., Sripa, B., Blacksell, S.D., Fenwick, S., & Thompson, R.C.A. (2012). Soil-transmitted helminthiasis in Laos: A community-wide cross-sectional study of humans and dogs in a mass drug administration environment. *American Journal of Tropical Medicine and Hygiene, 86*(4), 624–634. https://doi.org/10.4269/ ajtmh.2012.11-0413

[25] Htun, L.L., Rein, S.T., Win, S.Y. *et al*. Occurrence of gastrointestinal helminths and the first molecular detection of *Ancylostoma ceylanicum*, *Trichuris trichiura*, and *Trichuris vulpis* in dogs in Myanmar. *Parasitol Research,120*, 3619–3624 (2021). https://doi.org/10.1007/s00436-021-07290-w

[26] Inpankaew, T., Traub, R., Thompson, R.C.A., & Sukthana, Y. (2007). Canine parasitic zoonoses in Bangkok temples. *Southeast Asian Journal of Tropical Medicine and Public Health, 38*(2), 247–255.

[27] Kladkempetch, D., Tangtrongsup, S., & Tiwananthagorn, S. (2020). *Ancylostoma ceylanicum*: The neglected zoonotic parasite of community dogs in Thailand and its genetic diversity among Asian countries. *Animals, 10*(11), 1–15. https://doi.org/ 10.3390/ani10112154

[28] Mahdy, M.A.K., Lim, Y.A.L., Ngui, R., Fatimah, M.S., Choy, S.H., Yap, N.J., Al-Mekhlafi, H.M., Ibrahim, J., & Surin, J. (2012). Prevalence and zoonotic potential of canine hookworms in Malaysia. *Parasites and Vectors, 5*(1), 1–7. https://doi.org/10.1186/1756-3305-5-88

[29] Ng-Nguyen, D., Hii, S.F., Nguyen, V.A.T., Van Nguyen, T., Van Nguyen, D., & Traub, R.J. (2015). Re-evaluation of the species of hookworms infecting dogs in Central Vietnam. *Parasites and Vectors, 8*(1), 4–9. https://doi.org/10.1186/s13071-015-1015-y

[30] Ngui, R., Lim, Y.A.L., Traub, R., Mahmud, R., & Mistam, M.S. (2012). Epidemiological and genetic data supporting the transmission of *Ancylostoma ceylanicum* among human and domestic animals. *PLOS Neglected Tropical Diseases, 6*(2), 1–7. https://doi.org/10.1371/ journal.pntd.0001522

[31] Niamnuy, N., Kaewthamasorn, M., Congpuong, K., Phaytanavanh, B., & Lohsoonthorn, V. (2016). Prevalence and associated risk factors of intestinal parasites in humans and domestic animals across borders of Thailand and Lao PDR: Focus on hookworm and threadworm. *Southeast Asian Journal of Tropical Medicine and Public Health, 47*(5), 901–911.

[32] Pumidonming, W., Salman, D., Gronsang, D., Abdelbaset, A.E., Sangkaeo, K., Kawazu, S.I., & Igarashi, M. (2016). Prevalence of gastrointestinal helminth parasites of zoonotic significance in dogs and cats in lower Northern Thailand. *Journal of Veterinary Medical Science, 78*(12), 1779–1784. https://doi.org/10.1292/jvms.16-0293

[33] Schär, F., Inpankaew, T., Traub, R. J., Khieu, V., Dalsgaard, A., Chimnoi, W., Chhoun, C., Sok, D., Marti, H., Muth, S., & Odermatt, P. (2014). The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. *Parasitology International, 63*(4), 597–603. https://doi.org/ 10.1016/J. PARINT. 2014.03.007

[34] Tun, S., Ithoi, I., Mahmud, R., Samsudin, N.I., Heng, C.K., & Ling, L.Y. (2015). Detection of helminth eggs and identification of hookworm species in stray cats, dogs and soil from Klang Valley, Malaysia. *PLOS One, 10*(12), 1–12. https://doi.org/10.1371/ journal.pone. 0142231

[35] Silver, Z.A., Kaliappan, S.P., Samuel, P., Venugopal, S., Kang, G., Sarkar, R., & Ajjampur, S.S.R. (2018). Geographical distribution of soil transmitted helminths.

PLOS Neglected Tropical Diseases, 12(1), 7–16. https://doi.org/ 10.1371/ journal.pntd. 0006153

[36] Mationg, M.L.S., Tallo, V. L., Williams, G.M., Gordon, C.A., Clements, A.C.A., McManus, D.P., & Gray, D.J. (2021). The control of soil-transmitted helminthiases in the Philippines: The story continues. *Infectious Diseases of Poverty, 10*(1), 1–26. https://doi.org/10.1186/s40249-021-00870-z

[37] Hasegawa, H., Modrý, D., Kitagawa, M., Shutt, K.A., Todd, A., Kalousová, B., Profousová, I., & Petrželková, K.J. (2014). Humans and great apes cohabiting the forest ecosystem in Central African Republic Harbour the same hookworms. *PLOS Neglected Tropical Diseases, 8*(3). https://doi.org/ 10.1371/ journal.pntd. 0002715

[38] Hasegawa, H., Shigyo, M., Yanai, Y., McLennan, M.R., Fujita, S., Makouloutou, P., Tsuchida, S., Ando, C., Sato, H., & Huffman, M.A. (2017). Molecular features of hookworm larvae (*Necator* spp.) raised by coproculture from Ugandan chimpanzees and Gabonese gorillas and humans. *Parasitology International, 66*(2), 12–15. https:// doi.org/10.1016/j.parint.2016.11.003

[39] Boyko, R.H., Marie Harrison, L., Humphries, D., Galvani, A.P., Townsend, J.P., Otchere, J., Wilson, M.D., & Cappello, M. (2020). Dogs and pigs are transport hosts of necator americanus: Molecular evidence for a zoonotic mechanism of human hookworm transmission in Ghana. *Zoonoses and Public Health, 67*(5), 474–483. https://doi.org/ 10.1111/zph.12708

[40] El-Seify, M.A., Marey, N.M., Satour, N., Elhawary, N.M., & Sultan, K. (2021). Prevalence and molecular characterization of *Toxocara cati* infection in feral cats in Alexandria City, Northern Egypt. *Iranian Journal of Parasitology, 16*(2), 270–278. https://doi.org/ 10.18502/ijpa.v16i2.6319

[41] Eslahi, A.V., Badri, M., Khorshidi, A., Majidiani, H., Hooshmand, E., Hosseini, H., Taghipour, A., Foroutan, M., Pestehchian, N., Firoozeh, F., Riahi, S.M., & Zibaei, M. (2020). Prevalence of *Toxocara* and *Toxascaris* infection among human and animals in Iran with meta-analysis approach. *BMC Infectious Diseases, 20*(1), 1–17. https://doi.org/ 10.1186/s12879-020-4759-8

[42] Sahu, S., Samanta, S., Sudhakar, N.R., Raina, O.K., Gupta, S.C., Maurya, P.S., Pawde, A.M., & Kumar, A. (2014). Prevalence of canine toxocariasis in Bareilly, Uttar Pradesh, India. *Journal of Parasitic Diseases, 38*(1), 111–115. https://doi.org/ 10.1007/s12639-012-0207-z

[43] Schwartz, R., Bidaisee, S., Fields, P.J., Macpherson, M.L.A., & Macpherson, C.N.L. (2022). The epidemiology and control of *Toxocara canis* in puppies. *Parasite Epidemiology and Control, 16*(December 2021), e00232. https:// doi.org/10.1016/j. parepi.2021.e00232

[44] Department of Health. (2018). *Field Health Services Information System: Annual Report 2018*. https://www.doh. gov.ph/publications

[45] Lee, R.M., Moore, L.B., Bottazzi, M.E., & Hotez, P.J. (2014). Toxocariasis in North America: A systematic review. *PLOS Neglected Tropical Diseases, 8*(8), e3116. https://doi.org/ 10.1371/ journal.pntd. 0003116

[46] Fajutag, A.J., & Paller, V.G. (2013). *Toxocara* egg soil contamination and its seroprevalence among public school children in Los Baños, Laguna, Philippines. *The Southeast Asian Journal of Tropical Medicine and Public Health, 44*(4), 551–560.

[47] Paller, V.G.V., & De Chavez, E.R.C. (2014). *Toxocara* (Nematoda: Ascaridida) and other soil-transmitted helminth eggs contaminating soils in selected urban and rural areas in the Philippines. *Scientific World Journal, 2014*. https://doi.org/10.1155 /2014/386232

[48] Krone, O., Guminsky, O., Meinig, H., Herrmann, M., Trinzen, M., & Wibbelt, G. (2008). Endoparasite spectrum of wild cats (*Felis silvestris* Schreber, 1777) and domestic cats (*Felis catus* L.) from the Eifel, Pfalz region and Saarland, Germany. *European Journal of Wildlife Research, 54*(1), 95–100. https://doi.org/10.1007/s10344-007-0116-0

[49] Barua, P., Musa, S., Ahmed, R., & Khanum, H. (2020). Commonly found zoonotic parasite species in dogs and cats from a prominent pet market of Dhaka, Bangladesh. *Annual Research & Review in Biology, 35*(1), 17-23. https:// doi.org/10.9734/arrb/2020/ v35i130176

[50] Fontanarrosa, M., Vezzani, D., Basabe, J., & Eiras, D. (2008). An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): Age, gender, breed, mixed infections, and seasonal and spatial patterns. *Veterinary Parasitology, 136*, 283–295. https://doi.org/10.1016/j.vetpar.2005.11.012

[51] Sowemimo, O.A., & Asaolu, S.O. (2008). Epidemiology of intestinal helminth parasites of dogs in Ibadan, Nigeria. *Journal of Helminthology, 82(*1), 89–93. https://doi.org/DOI: 10.1017/ S0022149X078 75924

[52] Katagiri, S., & Oliveira-Sequeira, T. (2008). Prevalence of dog intestinal parasites and risk perception of zoonotic infection by dog owners in São Paulo State, Brazil. *Zoonoses and Public Health, 55*, 406–413. https://doi.org/10.1111/j.1863-2378.2008. 01163.x

[53] Awoke, E., Bogale, B., & Chanie, M. (2011). Intestinal nematode parasites of dogs: Prevalence and associated risk factors. *Internationa Journal of Animal and Veterinary Advances, 3.* (5): 374-378.

[54] Habluetzel, A., Traldi, G., Ruggieri, S., Attili, A.R., Scuppa, P., Marchetti, R., Menghini, G., & Esposito, F. (2003). An estimation of *Toxocara canis* prevalence in dogs, environmental egg contamination and risk of human infection in the Marche Region of Italy. *Veterinary Parasitology, 113*(3), 243–252. https://doi.org/10.1016/S0304-4017(03) 00082-7

[55] Little, S.E., Johnson, E.M., Lewis, D., Jaklitsch, R.P., Payton, M.E., Blagburn, B. L., Bowman, D.D., Moroff, S., Tams, T., Rich, L., & Aucoin, D. (2009). Prevalence of intestinal parasites in pet dogs in the United States. *Veterinary Parasitology, 166*(1), 144–152.https://doi.org/https://doi.org/10.1016/j.v etpar. 2009.07.044

[56] Overgaauw, P., & van Knapen, F. (2012). Veterinary and public health aspects of *Toxocara* spp. *Veterinary Parasitology,* 193. https://doi.org/10.1016/j.vetpar.2012.12.035

[57] Galgamuwa, L.S., Wickramasinghe, H., & Iddawela, D. (2020). Canine intestinal parasitic infections and soil contamination by *Toxocara* spp. in selected areas of Sri Lanka. *Tropical Parasitology, 10*(2), 114–119. https://doi.org/ 10.4103/tp.TP_62_19

[58] Chalkowski, K., Wilson, A.E., Lepczyk, C.A., & Zohdy, S. (2019). Who let the cats out? A global meta-analysis on risk of parasitic infection in indoor versus outdoor domestic cats (*Felis catus*). *Biology Letters, 15*(4). https://doi.org/10.1098 /rsbl.2018.0840

[59] Daryani, A., Sharif, M., Amouei, A., & Gholami, S. (2009). Prevalence of *Toxocara canis* in stray dogs, northern Iran. *Pakistan Journal of Biological Sciences: PJBS, 12*(14), 1031–1035. https://doi.org/10.3923/pjbs. 2009.1031.1035

[60] Yang, Y., & Liang, H. (2015). Prevalence and risk factors of intestinal parasites in cats from china. *BioMed Research International, 2015*, 1–6. https://doi.org/10.1155/2015 /967238

[61] Kantarakia, C., Tsoumani, M.E., Galanos, A., Mathioudakis, A.G., Giannoulaki, E., Beloukas, A., & Voyiatzaki, C. (2020). Comparison of the level of awareness about the transmission of echinococcosis and toxocariasis between pet owners and non-

pet owners in Greece. *International Journal of Environmental Research and Public Health, 17*(15), 5292. https://doi.org/10.3390/ijerph17155292

[62] San Jose, R., Magsino, P.J., & Bundalian, R. (2019). Pet owners' awareness on RA 9482 (Anti-Rabies Act of 2007) in Magalang, Pampanga, Philippines. *Heliyon, 5*(5), e01759.https://doi.org/10.1016/j.heliyon.2019 e01759

[63] San Jose, R.D., Magsino, P.J.P., & Bundalian, R.D.L. (2020). Factors affecting the knowledge, attitude, and practices of pet owners on responsible pet ownership in Magalang, Pampanga, Philippines: A cross-sectional study. *Philippine Journal of Veterinary Medicine, 57(*2), 182–195.