Parasites Hosts Dis 2025;63(2):182-187
https://doi.org/10.3347/PHD.24061
pISSN 2982-5164 • eISSN 2982-6799

Brief Communication

Prevalence of parasitic infections in stray cats from Gimpo-si, Gyeonggi-do, Korea

Sooji Hong[1,2], Hyejoo Shin[1,2], Seungwan Ryoo[1,2], Chung-Won Lee[3], Jae-Young Park[1,2], Jong-Yil Chai[4], Bong-Kwang Jung[1,2,*]

[1]MediCheck Research Institute, Korea Association of Health Promotion, Seoul 07572, Korea; [2]KAHP Parasite Museum, Korea Association of Health Promotion, Seoul 07572, Korea; [3]Sion Animal Hospital, Seoul 07677, Korea; [4]Department of Tropical Medicine and Parasitology, Seoul National University College of Medicine, Seoul 03080, Korea

Received: 23 August 2024
Accepted: 5 March 2025

***Correspondence**
mulddang@snu.ac.kr

Citation
Hong S, Shin H, Ryoo S, Lee CW, Park JY, Chai JY, Jung BK. Prevalence of parasitic infections in stray cats from Gimpo-si, Gyeonggi-do, Korea. Parasites Hosts Dis 2025;63(2):182-187.

Abstract

Stray cats serve as reservoir hosts for various zoonotic parasites, posing a significant risk of transmission to humans. This study aimed to assess the prevalence of parasitic infections in stray cats from Gimpo-si, Gyeonggi-do, Korea. A total of 101 fecal and 237 blood (serum) samples were collected from 237 stray cats captured through the trap-neuter-return program in 2021. The samples were analyzed using microscopy, nested-PCR, and ELISA to detect parasitic infections. Fecal examination revealed that *Toxocara cati* eggs were present in 26.7% (27/101) of samples, while eggs of *Spirometra* sp. (2%), *Clonorchis sinensis* (1%), and *Trichuris* sp. (1%) were also detected. PCR analysis identified *Toxoplasma gondii* DNA in 17 (16.8%) fecal samples, while genetic markers of *Cryptosporidium felis* and *Enterocytozoon bieneusi* were each detected in 4 (4%) samples. Positive rates of IgM and IgG were 21.9% (52/237) and 21.1% (50/237) in serological tests for *T. gondii*-specific antibodies. This study confirms the widespread presence of zoonotic parasites in stray cats from Gimpo-si, highlighting the potential public health risks associated with these infections. Continuous surveillance and control measures are essential from a One Health perspective to reduce the risk of zoonotic transmission.

Keywords: Stray cat, zoonotic pathogens, intestinal helminths, protozoa, prevalence

In recent years, pet ownership in Korea has increased, with more households owning cats and a significant rise in stray cat adoptions [1]. Stray cats often live in unsanitary environments, raising concerns about their exposure to infectious agents and the potential transmission of diseases to humans [2]. As human interactions with stray cats become more frequent, understanding their infectious status as potential carriers of zoonotic pathogens is crucial.

Feline parasitic infections are primarily caused by helminths such as *Toxocara cati* and *Ancylostoma* sp., as well as protozoan parasites like *Toxoplasma gondii* and *Giardia* sp. [2]. Additionally, various zoonotic intestinal trematodes have been identified in stray cats living in riverside areas of Korea [3]. However, direct transmission of these zoonotic pathogens from cats to humans has not been clearly established. Research on zoonotic parasite infections in stray cats is essential for understanding transmission dynamics and their significance from a One Health perspective. This study aimed to assess the prevalence of intestinal parasitic infections, with a particular focus on zoonotic pathogens, among stray cats in Gimpo-si (city), Gyeonggi-do (province), Korea.

All animal experiments were approved by the Institutional Animal Care and Use Com-

Author contributions
Conceptualization: Hong S, Chai JY, Jung BK
Data curation: Hong S, Shin H, Ryoo S, Lee CW, Park JY, Jung BK
Formal analysis: Hong S
Investigation: Hong S, Shin H, Ryoo S, Lee CW, Jung BK
Methodology: Hong S, Lee CW
Project administration: Park JY, Jung BK
Visualization: Hong S, Jung BK
Writing – original draft: Hong S, Jung BK
Writing – review & editing: Hong S, Chai JY, Jung BK

Conflict of interest
The authors declare no conflict of interest related to this study.

ORCID
Sooji Hong
https://orcid.org/0000-0002-8201-489X
Hyejoo Shin
https://orcid.org/0000-0002-1969-3586
Seungwan Ryoo
https://orcid.org/0000-0001-9916-6534
Chung-Won Lee
https://orcid.org/0009-0000-0576-5932
Jae-Young Park
https://orcid.org/0009-0006-6925-4254
Jong-Yil Chai
https://orcid.org/0000-0002-8366-0674
Bong-Kwang Jung
https://orcid.org/0000-0001-7581-3899

mittee of the Korea Association of Health Promotion under approval number KAHP-IACUC-2021001. A total of 237 stray cats from Gimpo-si, Gyeonggi-do, Korea, were captured between August and November 2021 as part of the government-operated trap-neuter-return program. The cats were transported to Sion Animal Hospital for neutering surgery. Blood samples were collected from all 237 cats during surgery, while 101 fecal samples were obtained from the cages of recovering cats. After the procedures, the cats were released back to their original locations. Of the 237 cats, 131 (55.3%) were female, and 106 (44.7%) were male. The collected fecal and blood samples were analyzed using stool examination, molecular techniques, and serological methods to determine the prevalence of parasitic infections in this study.

A total of 101 fecal samples were processed using the formalin-ether sedimentation method, with slight modifications based on the World Health Organization standard guidelines. The samples were examined under a microscope for helminth eggs. First, 1 g of fecal sample was mixed with 10–15 ml of water to create a suspension. This suspension was filtered through a funnel lined with 2–3 layers of gauze into a 15 ml tube. The tube was then centrifuged at 1,500 rpm for 2 min to sediment the helminth eggs. The supernatant was discarded, and the sediment was resuspended in water. This centrifugation process was repeated 3–5 times until the supernatant became clear. After the final centrifugation, the supernatant was discarded, and 10 ml of 10% formalin was added to the tube to mix with the sediment and fix the helminth eggs for 5 min. Then, 3 ml of ether was added, and the mixture was vigorously shaken for 1 min before being centrifuged at 3,000 rpm again for 5 min. The upper debris layer was carefully removed with a wooden applicator along with the supernatant. The remaining sediment was resuspended and examined microscopically. During optical microscopy, the initial observation of helminth eggs was conducted at ×100 magnification, followed by a closer examination at ×400 magnification to assess the size and morphology. The size of the detected helminth eggs was measured using an ocular micrometer.

DNA was extracted from 101 fecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA concentration and purity were measured using a NanoDrop One spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the DNA was stored at -80°C until testing. The DNA extracted from 101 fecal samples was analyzed for protozoan infections using nested-PCR. In this method, the first round of PCR amplification was performed using outer primers, followed by a second round with an inner primer set that binds within the previously amplified region, enhancing specificity and sensitivity. Different primers and PCR conditions were applied for each of the 5 protozoan species: *Blastocystis* spp., *Cryptosporidium felis*, *Enterocytozoon bieneusi*, *Giardia* sp., and *T. gondii* (Supplementary Table S1). The second-round PCR products were run on a 1.5% gel for 30 min, and the presence of DNA amplification bands at expected positions was confirmed using a ChemiDoc Imaging System (Bio-Rad, Hayward, CA, USA). Samples with bands at expected positions underwent Sanger sequencing at Macrogen (Seoul, Korea) for final confirmation. Serum was separated from 237 blood samples and tested for *T. gondii*-specific antibodies (IgM and IgG) using an ELISA. To separate serum, blood collected in serum-separating tubes was centrifuged at 3,000 rpm for 15 min. The serum was stored in 1.5 ml tubes at -20°C until testing. The ID Screen

Toxoplasmosis Indirect MultiSpecies Kit (IDvet, Montpellier, France) was used according to the manufacturer's instructions. The optical density (OD) of the samples was corrected by subtracting the OD of the negative control, and results were normalized by dividing by the OD of the positive control. Samples were considered negative if the corrected OD was below the threshold and positive if it met or exceeded the specified cutoff value.

The positive rates of intestinal helminths, protozoa, and *T. gondii*-specific antibodies in stray cats were analyzed using the Chi-square test.

A total of 30 fecal samples (29.7%) tested positive for 4 species of helminth eggs: *T. cati*, *Spirometra* sp., *Clonorchis sinensis*, and *Trichuris* sp. (Table 1; Fig. 1). Among these, *T. cati* had the highest positive rate at 26.7% (27/101). *Spirometra* eggs were found in 2% (2/101), while *C. sinensis* and *Trichuris* eggs were each detected in 1% (1/101). Out of 101 fecal samples analyzed using nested-PCR and confirmed by Sanger sequencing, 16.8% (17/101) were tested positive for *T. gondii*, while *C. felis* and *E. bieneusi* were each detected in 4% (4/101) (Table 1). *E. bieneusi*-positive samples were distributed across different regions. In contrast, *Giardia lamblia* and *Blastocystis* spp. were not detected by molecular methods.

A total of 237 serum samples were tested for *T. gondii*-specific antibodies. The results showed that 21.9% (52/237) tested positive for IgM, 21.1% (50/237) tested positive for IgG, and 28.7% (68/237) tested positive for either IgM or IgG (Table 1). Statistical analysis showed no significant difference in *T. gondii*-specific antibody results based on sex and weight (data not shown). However, when analyzing the geographical distribution of *T. gondii*-infected stray cats, a high prevalence was observed in a specific region (Gochone-eup).

In this study, we investigated the prevalence of intestinal helminths and protozoan parasites in stray cats from Gimpo-si. Fecal analysis identified 4 species of helminth eggs: *T. cati*, *Spirometra* sp., *C. sinensis*, and *Trichuris* sp. (Fig. 1; Table 1). Additionally, molecular and serological analyses provided significant insights into the prevalence of protozoan parasites, specifically *T. gondii*, *C. felis*, and *E. bieneusi* in stray cats (Table 1).

T. cati is a common zoonotic parasite that can cause eosinophilia and various syndromes in humans [4]. Infection occurs through the accidental ingestion of infectious eggs from the environment or the consumption of undercooked meat from infected intermediate

Table 1. Positive rates of parasites in 101 fecal and 237 blood samples of stray cats by morphological, molecular genetical, and serological examinations

Methods	Samples	Parasite species	No. (%)
Microscopic examination	Feces	*Toxocara cati*	27 (26.7)
		Spirometra sp.	2 (2.0)
		Clonorchis sinensis	1 (1.0)
		Trichuris sp.	1 (1.0)
Molecular examination		*Toxoplasma gondii*	17 (16.8)
		Cryptosporidium felis	4 (4.0)
		Enterocytozoon bieneusi	4 (4.0)
		Giardia lamblia	0 (0)
		Blastocystis sp.	0 (0)
ELISA	Blood	*T. gondi* (IgM)	52 (21.9)
		T. gondii (IgG)	50 (21.1)
		T. gondii (IgM or IgG)	68 (28.7)

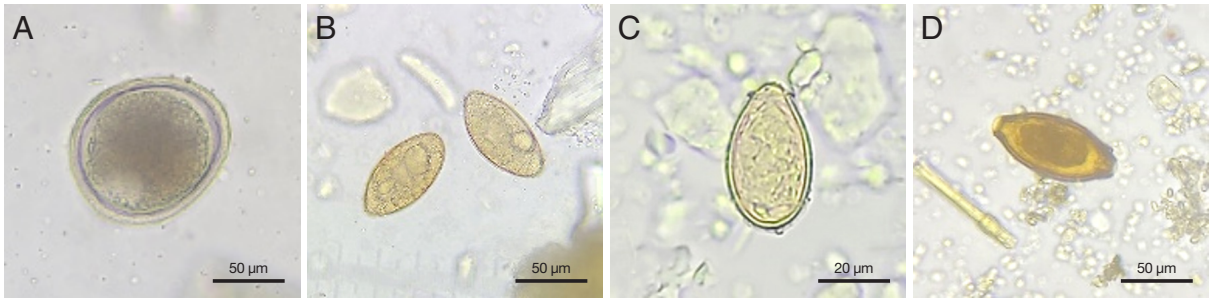

Fig. 1. Morphological features of helminth eggs in the feces of stray cats on microscopic examination. (A) *Toxocara cati*, (B) *Spirometra* sp., (C) *Clonorchis sinensis*, (D) *Trichuris* sp.

hosts [4]. This study confirmed that the positive rate of *T. cati* at 26.7% (27/101) was comparable to a previous survey result, which was 25.1% (102/407) in Daegu [3]. The epidemiological features of *T. cati* show that the higher prevalence in stray cats (28.6%) compared to household cats (11.6%) [4]. Given these findings, continuous control measures are necessary to mitigate potential health risks and prevent further transmission, particularly among stray cat populations.

Among intestinal helminths, *Spirometra* species found in cats pose significant public health concerns. Human sparganosis primarily occurs through the consumption of raw or undercooked meat from second intermediate hosts or by drinking untreated water contaminated with infected copepods [5]. Since cats and dogs serve as definitive hosts for *Spirometra* [5], ongoing monitoring and management strategies for *Spirometra* infection in natural hosts are crucial. Identifying *Spirometra* eggs based solely on morphology is challenging; therefore, DNA-based analysis is essential for accurate taxonomic classification [6]. In this study, the exact species of *Spirometra* could not be determined but is likely *Spirometra decipiens* or *Spirometra erinaceieuropaei*, both of which have been genetically identified in domestic cats in Korea [6].

Trichuris sp. is a common nematode in cats in Korea with zoonotic potential, meaning it can impact human health through environmental contamination or direct contact. Infections caused by *Trichuris* sp. are often asymptomatic or subclinical, with cats typically acquiring the infection through contaminated food or water. In this study, the positive rate was found to be 1%, which is lower than the 4.3% reported in a 2012 survey conducted in Suwon [7]. However, continued surveillance of stray cat populations is essential to prevent the re-emergence of trichuriasis. Unlike *T. cati*, *C. sinensis* has humans as its definitive host, with cats acting as reservoir hosts that contribute to environmental spread [8]. Infection with *C. sinensis* occurs through the consumption of raw freshwater fish containing *C. sinensis* metacercariae [9]. The *C. sinensis* positive-stray cat in this study was captured approximately 2 km from a tributary of the Han River, suggesting that the cat may have consumed infected fish, providing a potential route of transmission. Our study, along with previous research conducted across 26 regions nationwide, identified a *C. sinensis* positivity rate of 1% in stray cats [10]. Given these findings, it is crucial to adopt a One Health approach and implement management strategies to address the role of stray cats as reservoir hosts. *T. gondii* is a protozoan parasite capable of infecting most warm-blooded animals,

including humans, with cats serving as the definitive hosts. Human infection with *T. gondii* can occur through multiple routes, including ingestion of contaminated food or water, contact with infected soil or cat feces, blood transfusion, organ transplantation, or vertical transmission from mother to fetus [11]. Infected humans can show a range of clinical manifestations, from asymptomatic cases to severe central nervous system involvement [11]. In 2008, the seropositive rate for *T. gondii* was 15.8% in stray cats from Seoul [12]. A nationwide study conducted between 2017 and 2019 found that the seroprevalence of *T. gondii* in stray cats was approximately 6 times higher than in household cats [13]. In this study, serological analysis revealed that a certain percentage of *T. gondii* showed that 28.7% (68/237) tested positive for either IgM or IgG. Notably, the prevalence rate in Gochon-eup was 91.7% (11/12), highlighting a distinct geographical distribution of *T. gondii* infections.

Our molecular analysis also provided significant insights into the prevalence of other protozoan parasites, specifically *C. felis* and *E. bieneusi*. A previous study on shelter cats from Jeju Island detected *C. felis* in 0.6% (1/158) of samples, while *E. bieneusi* was found in 3.8% (6/158) of cases [14]. In comparison, our study on stray cats demonstrated a higher prevalence of *C. felis* (4%) and a comparable prevalence of *E. bieneusi* (4%), suggesting potential regional variations in infection rates.

This study was conducted on cats captured for neutering surgery, which presents a limitation, as the results may not fully represent the overall infection status of cats in Gimpo-si. However, this research provides valuable insights into parasitic infections in stray cats within the metropolitan area, consistent with previous findings from Suwon [7]. Another limitation is that the formalin-ether method used in this study is particularly effective for detecting parasite eggs with higher specific gravity. Consequently, eggs of parasites with varying sizes and densities, such as hookworms, *Strongyloides stercoralis*, *Fasciola* sp., and *Cytoisospora* sp., which have lower specific gravity, may have been underestimated in terms of true infection prevalence [15]. Future studies should employ alternative diagnostic techniques, such as more sensitive flotation methods or molecular tools, to improve accuracy in detecting infections.

In this study, we confirmed a high prevalence of zoonotic parasite infections in stray cats from Gimpo-si, near Seoul. These findings provide comprehensive data on parasitic infections in stray cats and offer essential insights for the prevention and management of zoonotic parasitic diseases. Future research should expand to include diverse regions and both stray and pet animals to further investigate zoonotic parasitic infections.

Acknowledgments

We appreciate the staff at Sion Animal Hospital, Seoul, Korea, who helped manage the stray cats.

Supplementary Information

Supplementary material is available with this article at https://doi.org/10.3347/PHD.24061.

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