

## Prevalence of gastrointestinal helminth parasites of zoonotic significance in dogs and cats in lower Northern Thailand

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**ABSTRACT.** Gastrointestinal zoonotic helminths of dogs and cats have a public health concern worldwide. We investigated the prevalence of gastrointestinal helminths of zoonotic significance in dogs and cats in lower Northern Thailand and utilized molecular tools for species identification of hookworms and *Opisthorchis viverrini*. Fecal samples of 197 dogs and 180 cats were collected. Overall prevalence of infection using microscopy was 40.1% in dogs and 33.9% in cats. Helminth infection found in both dogs and cats included hookworms, *Spirometra* spp., *Taenia* spp., *Toxocara* spp., *O. viverrini*, *Strongyloides* spp. and *Trichuris* spp. Hookworms were the most common helminth in dogs, while *Spirometra* spp. were the most prevalent in cats. Among hookworm infection in dogs and cats, *Ancylostoma ceylanicum* was the most prevalent hookworm, being 82.1% in hookworm infected dogs and 95.8% in hookworm infected cats. Mixed-infection due to hookworms and *Spirometra* spp. was the most dominant in both dogs and cats. Our finding showed that zoonotic helminth infection is highly prevalent in dogs and cats in the lower Northern area of Thailand.

**KEY WORDS:** cat, dog, gastrointestinal helminth, Thailand, zoonotic parasite

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Dogs and cats play a significant role as reservoir hosts for gastrointestinal zoonotic parasites including protozoa, trematode, cestode and nematode [7, 22, 24]. Humans can be infected via contact with a dog or cat or via contamination of infective stages in food or water [20, 35]. Worldwide, there is a significant variation in the prevalence of gastrointestinal zoonotic helminths in dogs and cats [19, 24]. High infection rates of zoonotic parasites including hookworms, *Trichuris* spp., *Spirometra* spp., *Taenia* spp., *Toxocara* spp. and *Opisthorchis* spp. have been reported [11, 17, 19, 24].

Infection of zoonotic helminths has previously been researched in Thailand. In the central area, a high prevalence of hookworm *Ancylostoma ceylanicum* was reported among dogs in temple communities in Bangkok [33]. The infections of zoonotic helminths, hookworms, *Trichuris* spp., *Toxocara* spp. and *Spirometra* spp. were found in dogs and cats in animal refuges [28]. In the Northeastern area, a high infection rate of liver fluke, *Opisthorchis viverrini* (*O. viverrini*) in

dogs and cats, was found in communities where *O. viverrini* infection in human was high [3].

In Thailand, infections of hookworms and *O. viverrini* are the major public health problems [15, 16, 25, 30, 33]. Infections of zoonotic hookworms, *A. ceylanicum* and *A. caninum*, have been reported in many areas [25, 33]. Molecular analysis showed *A. ceylanicum* is prevalent in humans and dogs in the Central and the Northeastern areas of Thailand [25, 33]. Prevalence and species of zoonotic hookworms in dogs and cats in the lower Northern area of Thailand are still unknown.

The infection of *O. viverrini* often coexists with minute intestinal flukes [9, 27]. Eggs of *O. viverrini* and minute intestinal flukes are similar in size and shape and are both operculated [32]. Microscopic examination for fecal eggs often leads to misdiagnosis [37]. PCR analysis with *O. viverrini* specific primers provides high sensitivity and specific results for the parasite detection [6, 18, 36]. In this study, dog and cat fecal samples were collected from communities where inhabitants originally migrated from the Northeastern area (pumidonming, interview), where *O. viverrini* is endemic [30]. Therefore, specific identification of *O. viverrini* infection in reservoir hosts around the communities at high risk of the infection is required for effective surveillance and control program.

Although surveys of zoonotic gastrointestinal helminths in dogs and cats had been done in Thailand, most of the

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studies have focused on the Central or Northeastern region [3, 12, 28, 33]. This was the first study to investigate prevalence of zoonotic helminth infection in dogs and cats in the lower Northern area of Thailand. In addition, this was the first study to identify species of hookworm infection in dogs and cats in the study area.

## MATERIALS AND METHODS

**Study area:** The study area is located in the lower Northern part of Thailand. Borders of the lower Northern Thailand connect to the upper Northern, the Central and the Northeastern parts and in addition connect to Lao People's Democratic Republic and Myanmar (Fig. 1). The climate is tropical and humid. The communities where samples were collected were characterized by poor hygiene. Over 50% of inhabitants in the communities originally migrated from the Northeastern area of Thailand (pumidonming, interview).

**Collection and examination of fecal samples:** Animal procedures were reviewed and approved by the Animal Research Ethics Committee of Naresuan University, Thailand. Fecal samples were collected from 197 dogs and 180 cats from the lower Northern region of Thailand between February and April 2014. Fecal samples were individually collected per rectum from each animal presented at the time of the investigation. General information including age, diet, defecation, vaccination and deworming were collected from dogs and cats owners. All dogs and cats used in this study showed asymptomatic of parasitic diseases. For microscopic examination, samples were processed by the sucrose flotation method [14] and formalin – ethyl acetate concentration technique (FECT) [1] at the Parasitology laboratory, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand. Helminth eggs were examined under a microscope at  $10 \times 10$  or  $10 \times 40$  magnifications.

**Identification of hookworm species:** DNA was extracted from 2 g of fecal samples using the following steps. Firstly, fecal samples were homogenized in the sucrose solution and centrifuged at 500 g for 10 min. Then, supernatant was harvested and diluted with water and centrifuged again at 2,500 g for 5 min at 4°C. The supernatant was discarded, and 100  $\mu$ l lysis buffer (600 mM EDTA, 1.3% (v/v) N-lauroylsarcosine and 2mg/ml Proteinase K) [38] was added to the pellet and subjected to 3–5 cycles of freezing at  $-80^{\circ}\text{C}$  for 20 min and thawing at  $98^{\circ}\text{C}$  for 1 hr in order to break hookworm eggs. Thereafter, 400  $\mu$ l CTAB buffer (2% (w/v) cetyl-trimethyl ammonium bromide, 1.4 M NaCl, 0.2% (v/v) mercapto-ethanol, 20 mM EDTA and 100 mM Tris (hydroxymethyl) aminomethane) [38] was added to the samples and incubated at  $70^{\circ}\text{C}$  for 1 hr. Then, phenol/chloroform extraction method was used to extract and purify DNA. The purified DNA was used for PCR with hookworm specific primers, RTHW1F (5'-GATGAGCATTGCWTGAATGCCG-3') and RTHW1R (5'-GCAAGTRCCGTTTCGACAAACAG-3') [33]. PCR products were then purified and sequenced. Cycle sequencing reactions were performed using a BigDye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems, Warrington, U.K.), and each sample was analyzed with an ABI

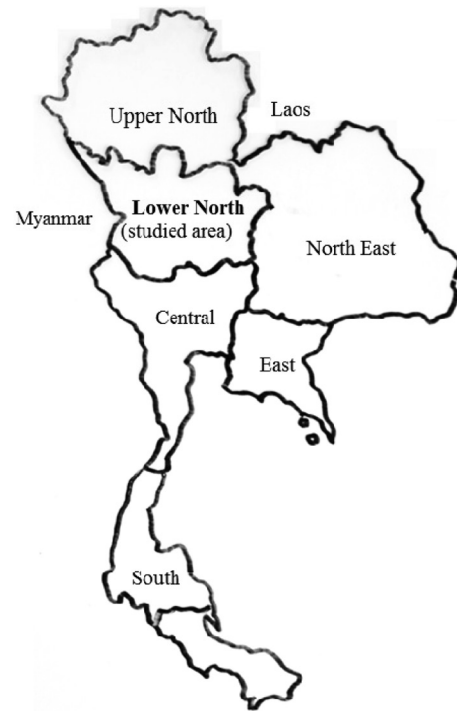


Fig. 1. Maps of Thailand showing the major regions and indicating the location of studied area.

PRISM 3100 Genetic Analyzer (Applied Biosystems). The obtained sequences were aligned and compared to published sequences of hookworm.

**Confirmation of *O. viverrini* infection:** 2 g of fecal samples with Opisthorchis-like egg were washed with the phosphate-buffered saline (PBS)-ethyl acetate concentration technique [6]. Then, 200 microliters of sediment of each purified positive fecal samples were processed according to manufacturer's instruction of QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). The purified DNA was used for PCR amplification with *O. viverrini* specific primers, OV-6F (5'-CTGAATCTCTCGTTTGT TCA-3') and OV-6R (5'-GTTCCAGGTGAGTCTCTCTA-3') [36].

## RESULTS

Out of 197 dog fecal samples examined by microscopy, 79 (40.1%) samples were positive for zoonotic significant gastrointestinal helminths. Among 197 samples, 57 (28.9%) were infected by one kind of helminth, while 22 (11.2%) were infected by a mixture of helminths (Table 1). The helminth infections found in samples were hookworms (21.3%), *Spirometra* spp. (15.2%), *Taenia* spp. (7.1%), *Toxocara* (3.6%), *O. viverrini* (3.0%) and *Strongyloides* spp. (1.5%) (Table 2 and Fig. 2A). Hookworms were the most prevalent, followed by *Spirometra* spp. Co-infections of hookworms or *Spirometra* spp. with other zoonotic helminth were common. Co-infection of hookworms and *Spirometra* spp. was the most prevalent (Table 3).

Table 1. Gastrointestinal parasitic infection in dogs and cats in lower Northern Thailand

Parasitic infection	No. (%) positive	
	Dog (n=197)	Cat (n=180)
Total infection	79 (40.1)	61 (33.9)
Infection with single species	57 (28.9)	49 (27.2)
Mixed infection	22 (11.2)	12 (6.7)

Out of 180 cat fecal samples examined microscopically, 61 (33.9%) cats were positive for helminth infection. Among all positive samples, 49 (27.2%) samples had infection from one kind of helminth, while 12 (6.7%) samples had mixed infections (Table 1). The helminths found were *Spirometra* spp. (20.0%), hookworms (13.9%), *Toxocara cati* (2.2%), *O. viverrini* (3.3%) and *Trichuris* spp. (0.6%) (Table 2 and Fig. 2B). *Spirometra* spp. were the most prevalent helminth. Co-infection of *Spirometra* spp. or hookworms with other helminths was common. Mixed infection of *Spirometra* spp. and hookworms was also the most common in cats (Table 3).

Molecular identification of hookworm infection in dog using PCR amplification with hookworm-specific primers yielded a specific PCR product size at 380 bp in 28 (14.2%) dog fecal samples. DNA sequences were successfully obtained from all 28 PCR positive samples. Sequences revealed 100% identity to *A. ceylanicum* strain GD-M55 (KF279136) in 23 (82.1%) samples. Another 5 (17.9%) samples showed 100% identity to *A. caninum* GD-M45 (KC755026) (Table 4).

For the cat samples, the PCR amplification with hookworm specific primers revealed positivity in 25 (13.9%) samples. DNA sequences were obtained from all 25 samples. BLAST results showed that 21 (84.0%) samples were 100% identity

Table 2. The prevalence (%) of infection of helminth parasite in 197 dogs and 180 cats from lower Northern Thailand

Helminth parasite	No. (%) positive	
	Dog	Cat
Hookworms	42 (21.3)	25 (13.9)
<i>Spirometra</i>	30 (15.2)	36 (20.0)
<i>Taenia</i>	14 (7.1)	0 (0.0)
<i>Toxocara</i>	7 (3.6)	4 (2.2)
<i>O. viverrini</i>	6 (3.0)	6 (3.3)
<i>Strongyloides</i>	3 (1.5)	0 (0.0)
<i>Trichuris</i>	0 (0.0)	2 (0.6)

to *A. ceylanicum* strain GD-M55 and 1 (4.0%) sample was 100% identity to *A. caninum* strain GD-M45. Three (12.0%) samples were infected with both *A. ceylanicum* strain GD-M55 and *A. ceylanicum* GD-M76 (Table 4).

The PCR analysis confirmed *O. viverrini* infection in 6 dog fecal samples and 6 cat fecal samples.

## DISCUSSION

Dogs and cats are important reservoir hosts of various zoonotic helminths [7, 22, 24], many of which cause serious public health problems. Here, we reported the prevalence of zoonotic intestinal helminths in lower Northern Thailand as 40.1% (79/197) in dogs and 33.9% (61/180) in cats, respectively. Zoonotic helminths found included hookworms, *Spirometra* spp., *Toxocara*, *O. viverrini*, *Taenia* spp., *Strongyloides* and *Trichuris* [29, 34].

Among zoonotic helminth infections in dogs in the lower Northern area of Thailand, hookworms were the most

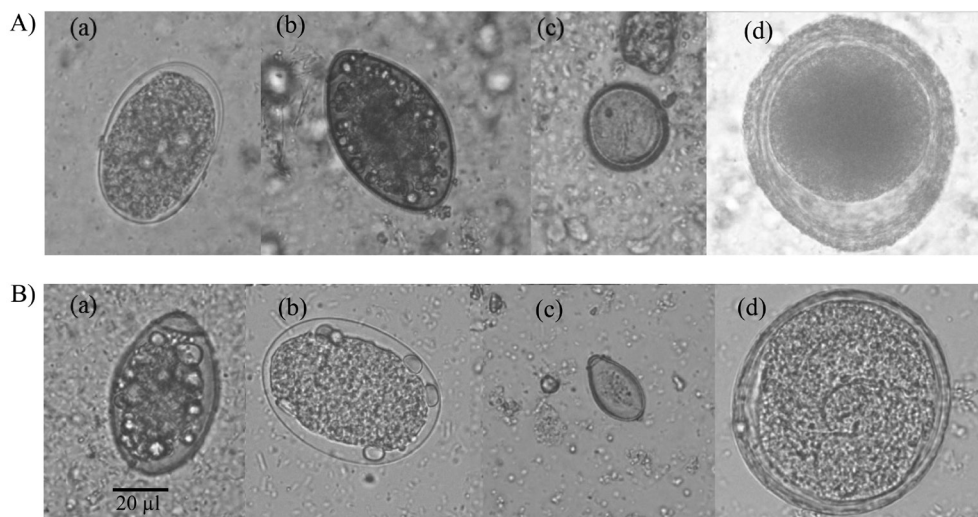


Fig. 2. Microscopic images of parasitic eggs found in dog fecal samples (A) which were hookworm (a), *Spirometra* (b), *Taenia* (c) and *Toxocara* (d), and in cat fecal samples (B) which were *Spirometra* (a), hookworm (b), *Opisthorchis viverrini* (c) and *Toxocara* (d).

Table 3. The prevalence (%) of mixed infection of helminth parasite in 197 dogs and 180 cats from the lower Northern Thailand

Mixed infection	No. (%) mixed infection	
	Dog	Cat
Hookworms/ <i>Spirometra</i>	10 (5.1)	6 (3.3)
Hookworms/ <i>Toxocara</i>	2 (1.0)	1 (0.6)
Hookworms/ <i>Ascaris</i>	2 (1.0)	0 (0.0)
Hookworm/ <i>O. viverrini</i>	2 (1.0)	0 (0.0)
<i>Spirometra</i> / <i>Taenia</i>	2 (1.0)	0 (0.0)
<i>Spirometra</i> / <i>O. viverrini</i>	2 (1.0)	3 (1.7)
<i>Spirometra</i> / <i>Toxocara</i>	1 (0.5)	2 (1.1)
<i>Toxocara</i> / <i>O. viverrini</i>	1 (0.5)	0 (0.0)

prevalent helminth, and *Spirometra* was the second most prevalent. Our results confirmed that hookworm infection in dogs is common in Thailand [12, 33]. The high prevalence of hookworm infections in dogs can contribute to the occurrence of zoonotic ancylostomiasis in human [11].

Zoonotic helminth infections in cats were different from dogs. *Spirometra* was the most prevalent, while hookworms were the second most prevalent helminth. High rates of *Spirometra* infection might be a reflection of the fact that most cats roam freely and had access to small prey as a food source. High infection rates of *Spirometra* spp. in cats might indicate a high infection rate of plerocercoid and plerocercoid in intermediate hosts in the area. The infection of *Spirometra* spp. in cats and dogs can lead to a high risk of sparganosis in humans who have the habit of eating undercooked meat [2, 4]. However, human sparganosis in Thailand is rare. In the period 1943–2010, only 53 cases had been reported [2, 4].

Co-infections of hookworms and *Spirometra* spp. were common in both dogs and cats. This finding indicated a higher risk level in the area of zoonotic diseases caused by the two parasites. However, zoonotic diseases caused by hookworms are more prevalent in Thailand [2, 11].

The larva migrans can also be caused by *Toxocara* [23] which were also found in feces of dogs and cats examined in this survey. Human can be infected by the ingestion of embryonated eggs that could be present in soil contaminated with dog or cat feces [12]. However, unlike hookworms and *Spirometra* spp., infection of *Toxocara* spp. in cats and dogs was low. This finding may be attributed to the hot climate of the area where fecal samples were collected. In summer, the lower Northern area is very dry and hot, sunlight is very strong, and the temperature reaches 40°C. Several studies reported that incubation of *Toxocara* egg at 37°C stops embryonation of the parasite egg [8, 21]. Possibly, hot and dry climate in the lower Northern area kills infective stage of *Toxocara* spp. which lead to low infection rate of the parasite in cats and dogs in the area.

Significant zoonotic hookworms include *A. ceylanicum*, *A. braziliensis* and *A. caninum* [10, 11, 25]. Molecular analysis revealed that the most prevalent hookworm (over 80%) found in dogs and cats in the lower Northern area was

Table 4. Identification of hookworm infections from 197 dogs and 180 cats using floatation, PCR and sequencing techniques

Technique/hookworm species	Positivity (%)	
	Dogs	Cats
Flotation technique	21.3	13.9
PCR	14.2	13.9
Sequencing	14.2	13.9
Hookworm species		
<i>Ancylostoma ceylanicum</i> GD-M55	82.1	84
<i>Ancylostoma caninum</i> GD-M45	17.9	4
<i>A. ceylanicum</i> GD-M55 and <i>A. ceylanicum</i> GD-M76	0	12

*A. ceylanicum*. *A. ceylanicum* is highly prevalent in many areas in Asian countries [29, 33, 34] and is known to produce potent infections in humans. *A. ceylanicum* is the second most common hookworm infection in humans that can lead to anemia [10, 11].

Zoonotic hookworm, *A. caninum*, was found to have low infection rates in both dogs and cats. Similar to other areas, prevalence of *A. caninum* was lower than that of *A. ceylanicum* [33, 34]. Although its infection rate was low, this hookworm can result in eosinophilic enteritis and chronic abdominal pain in human [5, 26]. Other zoonotic hookworm, such as *A. braziliensis*, was not found in this area.

*O. viverrini* is a significant zoonotic infection in Thailand [30]. Microscopic examination for fecal eggs leads to misdiagnosis of *O. viverrini* egg [37]. Therefore, in this study, molecular analysis was applied to identify *O. viverrini* infection in both cats and dogs. PCR analysis with *O. viverrini* specific primers [36] confirmed *O. viverrini* infection in dogs and cats in the lower Northern area. The infection rate was relatively less than the rate in the Northeastern region where *O. viverrini* infection is highly prevalent [30]. However, it is important to emphasize that over 50% of inhabitants in these communities originally migrated from the Northeastern area (pumidonming, interview). This could be the reason behind the existence of *O. viverrini* infection in dogs and cats in the studied area. Dogs and cats could potentially be infected through ingestion of raw or undercooked cyprinoid fish which is infected with *O. viverrini* metacercariae. The infection in dogs and cats can increase the occurrence of infected snails and cyprinoid fish which consequently increase the incidence of *O. viverrini* infection in humans.

PCR and sequencing were used for detection and identification of parasites in various specimens with high sensitivity and specificity [33, 36]. In our survey, molecular analysis was applied for two significant helminths infection, hookworms and *O. viverrini*. Morphological identification of hookworm larvae or eggs to species is difficult, and molecular identification provides great results in this regard [34]. PCR amplification with *O. viverrini* specific primers (OV-F and OV-R) was used to confirm *O. viverrini* infection. The primers are highly specific to *O. viverrini*. No false-positive amplification was observed when testing with DNA of min intestinal flukes, such as *Centrocestus* spp. and *Haplorchis taichui* or other trematode parasites, such as *Fasciola gigantica*.



*tica* and *Paragonimus heterotremus* [13, 36]. However, inhibitors present in fecal samples can inhibit PCR reaction as shown in many epidemiological studies including our study where PCR analysis failed to yield positive results in some microscopic positive samples [29, 31].

Dogs and cats with low intensity of helminth infection are often asymptomatic, however, adult worms still produce eggs, being shed out with feces. Dogs and cats in the lower Northern area roam around freely. Free roaming of infected cats and dogs causes contamination of zoonotic parasites to the environment [5, 35].

In conclusion, we report the prevalence of gastrointestinal helminths of zoonotic significance in dogs and cats in lower Northern Thailand. Variable helminths were detected, namely hookworms, *Spirometra* spp., *Taenia* spp., *Toxocara* spp., *O. viverrini*, *Strongyloides* spp. and *Trichuris* spp. Hookworm infections included *A. ceylanicum* and *A. caninum*, and *A. ceylanicum* was the most prevalent hookworm found in dogs and cats in the lower Northern area of Thailand.

The zoonotic helminth infections in dogs and cats in the lower Northern Thailand are considered neglected, because relatively little attention has been devoted to their surveillance, prevention and treatment.

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