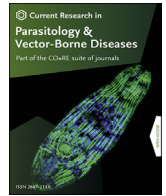


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Helminth infections in dogs in Phu Tho Province, northern Vietnam

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ABSTRACT

Helminths are among the most-commonly encountered disease-causing agents in dogs all over the world, including Vietnam. A cross-sectional survey was carried out between March and December 2019 in four dog slaughter-slabs in Yen Lap District, Phu Tho Province, northern Vietnam, aiming to reveal the occurrence of gastrointestinal helminths in dogs. Worms were collected from 350 dog samples and identified by morphological techniques followed by molecular methods for tapeworms and hookworms. Nine species, including *Taenia hydatigena*, *Spirometra erinaceieuropaei*, *Dipylidium caninum*, *Spirocerca lupi*, *Ancylostoma ceylanicum*, *Ancylostoma caninum*, *Toxocara canis*, *Toxascaris leonina* and *Trichuris vulpis*, were found to occur in this area. Infection with at least one worm species was found in 272 dogs (77.7%; 95% Wilson score CI: 73.1–81.8%). The most common species was *A. ceylanicum*, with a prevalence of 45.1% (95% Wilson score CI: 40.0–50.4%) while *T. hydatigena* had a very low prevalence of 0.3% (95% Exact CI: 0.0–1.6%). Co-infection with hookworms and *D. caninum* was common. Five of the recovered helminth species have a zoonotic potential. Control of these parasites is necessary in order to protect human and animal health.

1. Introduction

Vietnam, a middle-income country in Southeast Asia, has a dog population of 7.7 million distributed over 3.8 million households (DAH, 2020). Dog keeping serves multiple purposes in Vietnam, including guarding the house, hunting, herding livestock and petting. In addition, consumption of dog meat is part of the Vietnamese traditional cuisine. Vietnamese dogs usually live in the vicinity of humans, but are also often left free-roaming, especially in rural and mountainous areas. Consequently, dogs come in contact with other domestic and wild animals such as pigs, cattle, poultry, cats, snakes and frogs, and the environment in which they live. Combined with poor hygienic conditions this creates opportunities for the transmission of helminths. Dogs serve either as definitive or reservoir hosts of many zoonotic parasite species, posing major public health,

economic, and social threats, particularly in developing countries such as Vietnam where the management of dogs is commonly not controlled (Carrique-Mas & Bryant, 2013).

In Vietnam, several helminth species have been recorded in the dog's gastrointestinal tract; these findings were mostly reported in the local literature (Thuy, 1996; Khuong, 2005; Lan et al., 2011; Bang et al., 2016) and generally do not include prevalence data. The most common method used to estimate the prevalence of gastrointestinal helminths in dogs is coprological examination for parasite eggs, which often does not allow identification to the species level and may lead to underestimation due to low sensitivity (Adolph et al., 2017; Byrne et al., 2018). The examination of the intestinal content at necropsy is more performant as it provides information on parasite numbers and allows for the morphological and molecular identification of the recovered worm species (Byrne et al., 2018).

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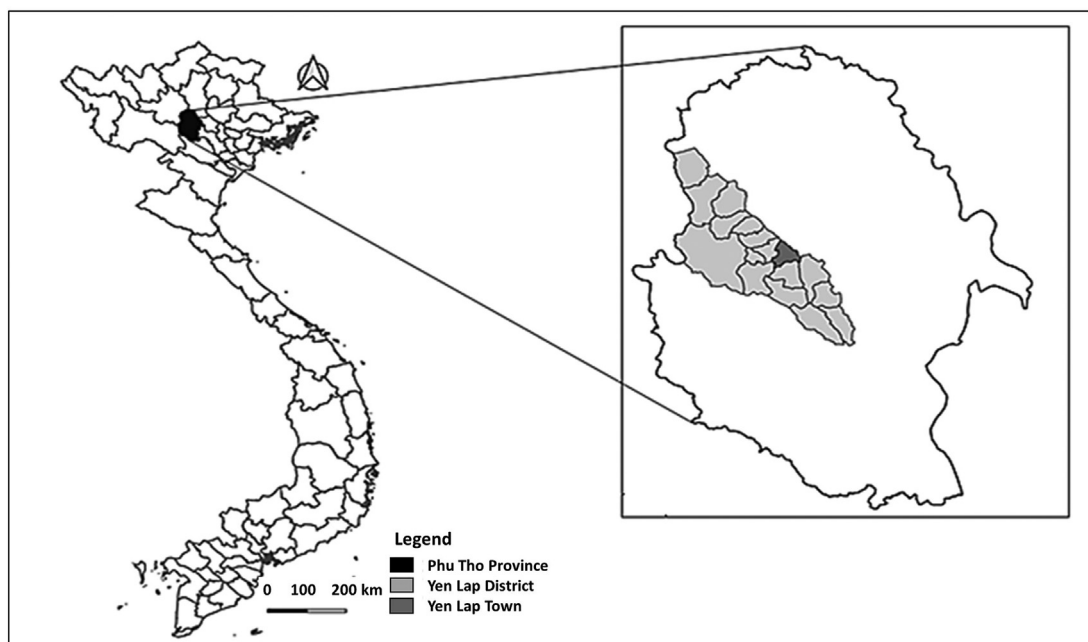


Fig. 1. Map of Vietnam showing Phu Tho Province (insert) and Yen Lap District (dark grey).

Therefore, we conducted a necropsy study on gastrointestinal helminths in dogs in Phu Tho Province in North Vietnam to identify and establish the prevalence of intestinal parasitic infections and to point out their zoonotic potential.

2. Materials and methods

A cross-sectional survey was carried out between March and December 2019 in dog slaughter-slabs in Yen Lap District, Phu Tho Province (21°15'45.01"N, 105°7'31.54"E) (Fig. 1). This province is located in the Northern Midlands region of Vietnam, around 120 km from Hanoi (Nguyen et al., 2020). The dog population in the province is around 260,000 (DAH, 2020). Dog meat is consumed as a traditional food (Tuyen, 2012).

In Yen Lap District, there are six dog slaughter slabs according to the District Station of Animal Health, each slab usually processes 1–2 dogs/day, rarely 3 or 4 dogs/day. Four slaughter-slab owners agreed to participate in this study. They were asked to collect the gastrointestinal tract (i.e. oesophagus to anus) and note information on each slaughtered dog: slaughtering date, dog weight and sex. All dogs were more than 6 months old. After collection, the gastrointestinal tract of each dog was transported to the laboratory in sealed plastic bags in a cool box, where they were immediately processed or kept at -20°C until further processing. Based on the prevalence of *Taenia hydatigena* in dogs found in a previous study conducted in this province (35%), and a desired precision of 5%, a sample size of 350 dogs was required (Martin et al., 1987; Lan et al., 2011).

The entire gastrointestinal (GI) tract from each dog was divided into five parts, which were processed separately: oesophagus and the stomach, three small intestine segments of equal length, and the large intestine. Each part of the GI tract was opened longitudinally (El-Shehaby et al., 1999). All visible gastrointestinal helminths were collected, rinsed in saline, and transferred to 70% ethanol for later identification. Next, the gastrointestinal content was removed, and the mucosa was washed with saline. The washings were collected and passed through a sieve of 250 μm mesh to remove large debris and to recover small helminths. All recovered helminths were washed and stored in 70% ethanol before identification (Dalimi et al., 2006; Adolph et al., 2017). After washing, the gastrointestinal mucosa was inspected for gross lesions.

Nematodes were fixed in glacial acetic acid (concentration: 100%) overnight for clearing up and stored in an ethanol glycerin solution (ratio: 7:1), before being identified following the keys to the nematode parasites of vertebrates (Gibbons, 2009) and morphological characteristics of hookworms (Hieu et al., 2016). Cestodes were fixed in an alcohol, formaldehyde, acetic acid (AFA) solution (ratio: 5:10:2); the scolex and proglottids were stained following the Semichon's acetocarmine stain method (Morgan & Hawkins, 1949). The cestodes were identified according to the keys and guidelines given by Khalil et al. (1994).

Hookworm and tapeworm species identification was confirmed by PCR due to the difficulty of distinguishing by morphology. DNA from two *T. hydatigena*, 10 *Spirometra* spp., 12 *Dipylidium caninum* and a random selection of hookworms from 50 dogs was extracted using the DNeasyBlood and Tissue Extraction Kit (Qiagen, Hilden, Germany). Identification of *D. caninum* was confirmed by using primers DC28S-1R and DC28S-1F targeting a 28S rDNA region in an adjusted amplification protocol (Beugnet et al., 2014). PCR targeting a mitochondrial 12S rDNA fragment, followed by RFLP was used to identify *Taenia* spp. (Rodriguez-Hidalgo et al., 2002; Devleeschauwer et al., 2013). Identification of *Spirometra erinaceieuropaei* was confirmed by using primers p1f and p1r, which amplified a 440-bp product of the *cox1* gene, following the protocol of Jeon et al. (2015). Amplification of the internal transcribed spacer (ITS1, 5.8S and ITS2) regions were utilized as genetic markers for *Ancylostoma* spp., using the primers RTGHF1/RTGHR1 (Traub et al., 2004), and RTGHF1/RTABCR1 (Ng-Nguyen et al., 2015). Confirmation of identification of hookworm species, *S. erinaceieuropaei* and *D. caninum* was achieved by sequencing of the PCR products at the Institute of Genome Research (Hanoi, Vietnam) and the VIB Genetic Service Facility (University of Antwerp, Belgium). The sequences were edited and aligned using BioEdit. BLAST was performed on NCBI, and sequences were compared with those deposited in GenBank.

All collected data were entered in a Microsoft Office Excel 2016 spreadsheet. A descriptive analysis of the occurrence and gross lesions was conducted. The prevalence of helminths and the associated 95% Wilson score confidence intervals (CIs) were calculated. In case of low case counts, the Clopper-Pearson exact CIs were calculated. All statistical analyses were conducted using R software (R Core Team, 2020).

Table 1
The occurrence of gastrointestinal helminths in dogs in Phu Tho Province

Species	No. of infected dogs	Prevalence (%)	95% CI	No. of worms (range)
Cestodes	67	19.1	15.4–23.6	
<i>Taenia hydatigena</i>	1	0.3	0–1.6	2
<i>Dipylidium caninum</i> ^a	62	17.7	14.1–22.1	2–>50
<i>Spirometra erinaceieuropaei</i> ^a	10	2.9	1.6–5.2	1–5
Nematodes	249	71.1	66.2–75.6	
<i>Spirocerca lupi</i>	12	3.4	2.0–5.9	2–8
<i>Ancylostoma</i> spp.	238	68.0	62.9–72.7	
<i>A. ceylanicum</i> ^a	158	45.1	40.0–50.4	2–>200
<i>A. caninum</i> ^a	113	32.3	27.6–37.4	2–>200
<i>Toxocara canis</i> ^a	21	6.0	4.0–9.0	1–26
<i>Toxascaris leonina</i>	14	4.0	2.4–6.7	1–15
<i>Trichuris vulpis</i>	2	0.6	0.2–2.1	4–5
Total infected dogs	272	77.7	73.1–81.8	–

Note: Total no. of sampled dogs: $n = 350$.

^a Potentially zoonotic helminths.

3. Results and discussion

A total of 350 adult dogs were sampled from four dog slaughter slabs in Phu Tho including 173 females and 177 males, and 311 dogs of indigenous breed and 39 cross-breeds. A total of 272 dogs were infected with gastrointestinal helminths, resulting in an overall prevalence of 77.7% (95% CI: 73.1–81.8%) (Table 1). This prevalence is lower than the 92.1% recorded in Hanoi city in northern Vietnam (Thuy, 1996) and 97.8% in 13 provinces in southern Vietnam (Khuong, 2005), but similar to a 73.6% prevalence found in An Giang Province in southern Vietnam (Bang et al., 2016). This high prevalence is likely due to the practice of allowing dogs to roam freely and the poor hygiene of the environment they live in.

Nine helminth species were identified of which five species have a zoonotic potential (Table 1) (Jacobs et al., 1997; Inpankaew et al., 2015). The recovered helminth species include three cestode species (*T. hydatigena*, *S. erinaceieuropaei* (GenBank: ON246997) and *D. caninum* (GenBank: ON248386) and six nematode species (*Spirocerca lupi*, *Ancylostoma ceylanicum* (GenBank: OL454900), *Ancylostoma caninum* (GenBank: ON340755), *Toxocara canis*, *Toxascaris leonina* and *Trichuris vulpis*).

Among the cestode species, *D. caninum* was the most common; 27 dogs carried more than 10 *D. caninum*. The low prevalence of *T. hydatigena* (0.3%) in the sampled dogs was somewhat unexpected as in the same province Lan et al. (2011) found a prevalence of 35% in dogs, while Nguyen et al. (2020) estimated the prevalence of *T. hydatigena* cysticercosis in the pig intermediate host at 18%. Generally, the prevalence of *Taenia* spp. is much higher in the intermediate than in the definitive hosts, which can be explained by the high number of eggs being shed in the environment by cestode definitive hosts (Featherston, 1969). Similarly, the prevalence of *T. hydatigena* in dogs and pigs in Laos was reported at 1.9% and 22.4%, respectively (Conlan et al., 2012). The finding of *S. erinaceieuropaei* confirms the presence of this cestode in Vietnam. This cestode has previously been found in pigs (Nguyen et al., 2020) and humans (Le et al., 2017) in Vietnam and is a cause of sparganosis in humans.

Hookworms were the most common nematodes found, including the zoonotic *A. ceylanicum* and *A. caninum*. The majority of dogs ($n = 131$) carried less than 50 hookworms, while 50 dogs were infected with 50–100 hookworms, and 46 dogs were infected with more than 100 hookworms. Lesions associated with hookworm infection were found mostly in the small intestine and consisted of hemorrhagic enteritis, ulcerations and a swollen intestinal mucosa (Fig. 2). The lesions were more severe in dogs with high numbers of hookworms. Although *A. ceylanicum* was detected earlier in Phu Tho Province (Linh et al., 2018), we have now confirmed its occurrence using a molecular technique. *Ancylostoma ceylanicum* in dogs and cats was also reported to be highly prevalent in other Asian countries including Thailand (67–77%), Malaysia (46–76%), Laos (69%) and China (33–51%) (Traub et al., 2008; Mahdy et al., 2012; Ngui et al., 2012; Fu et al., 2019; Kladkempetch et al., 2020). In Vietnam, this species was previously reported in dogs in Hanoi (North Vietnam) and Daklak Province (Central Vietnam), and in humans in Long An Province (South Vietnam) (Ng-Nguyen et al., 2015; Hieu et al., 2016; Linh et al., 2021). Therefore, *A. ceylanicum* can be considered endemic in Vietnam, posing a human health risk. Furthermore, twelve dogs were infected with *S. lupi* which were observed to locate within 1–2.5 cm nodules in the oesophagus.

Overall, co-infections were found in 94 dogs, with dogs carrying two ($n = 70$), three ($n = 21$) or four ($n = 3$) helminth species (Table 2). Co-infection with hookworms and *D. caninum* was most common. In comparison to this study, more dogs in Canada, Hungary and Pakistan were

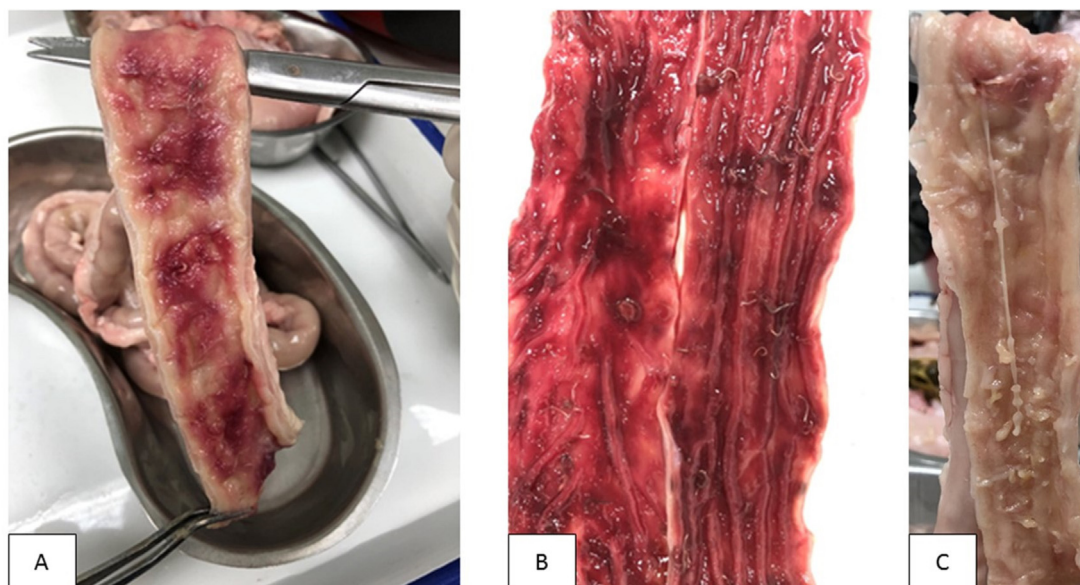


Fig. 2. Gross pathological lesions caused by helminth infections in dogs sampled in northern Vietnam. A Ulceration and hemorrhagic enteritis caused by hookworms. B Severe hemorrhagic enteritis caused by hookworms. C Ulceration and a swollen intestinal mucosa.

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