Helminth infections in dogs in Phu Tho Province, northern Vietnam

Thi Thuy Man Nguyen¹, Pierre Dorny², The Dung Dinh³, Van Toan Nguyen⁵, Hong Nhan Nguyen⁴, Thi Giang Thanh Nguyen⁶, Ha Thanh Dao⁷, Veronique Dermauw⁸,*

¹ National Center for Veterinary Diagnosis, Hien Ninh, Soc Son, Hanoi, Vietnam
² Institute of Tropical Medicine, Department of Biomedical Sciences, Nationalestraat 155, 2000 Antwerpen, Belgium
³ Ghent University, Faculty of Veterinary Medicine, Salisburylaan 133, 9820, Merelbeke, Belgium
⁴ Vietnamese-Russian Tropical Center, 63 Nguyen Van Huyen, Nghia Do, Cau Giay, Hanoi, Vietnam
⁵ National University of Agriculture, Faculty of Veterinary Medicine, Tran Quy, Hanoi, Vietnam
⁶ National Institute of Veterinary Research, 86 Truong Chinh, Dong Da, Hanoi, Vietnam

ARTICLE INFO

Keywords:
Dog
Vietnam
Gastrointestinal helminths
Prevalence

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).

* Corresponding author.
E-mail address: vdermauw@itg.be (V. Dermauw).

https://doi.org/10.1016/j.crpvbd.2022.100091
Received 7 March 2022; Received in revised form 29 April 2022; Accepted 7 May 2022
2667-114X/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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-Shehabi 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 (Hier 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 *Spiromera* 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; Devleesschauwer 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 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).
Severe hemorrhagic enteritis caused by hookworms. To a 73.6% prevalence found in An Giang Province in southern Vietnam (Khuong, 2005), but similar findings have been reported in 13 provinces in southern Vietnam (Traub et al., 2008). Gross pathological lesions caused by helminth infections in dogs sampled in northern Vietnam (Nguyen et al., 2020). 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 infected with hookworms and A. caninum.
found to have co-infections with two helmint species, while in Tanzania and Australia co-infections were less common (Khan et al., 2020). Finally, no trematode species were detected, while in Nam Dinh Province 56.9% of dogs were found infected with fish-borne trematodes, including Clonorchis sinensis and minute intestinal flukes (Nguyen et al., 2009). Regional differences in trematode species distribution can be due to different environmental conditions and dog feeding habits.

4. Conclusion

This study has recorded a high prevalence of helmint infections in dogs in Phu Tho, northern Vietnam. The recovered helmint species may have pathological consequences in dogs and/or pose a zoonotic risk. Therefore, helmint control programmes in dogs should be developed to reduce the risks of human infection and improve the health of the dog population.

Funding

This work was supported by the Directorate General for Development (DGD), Belgium, through the individual PhD program (Thi Thuy Man Nguyen) of the Institute of Tropical Medicine Antwerp, Belgium.
Gibbons, L.M., 2009. Keys to the nematode parasites of vertebrates: Supplementary volume. CAB International, Wallingford.

Hieu, D.D., Linh, B.K., Linh, N.V., Phong, V.T., Anh, L.T.L., Hai, V.V., Long, S.T., 2016. Distinct morphological characteristics of zoonotic canine hookworm Ancylostoma ceylanicum circulating in dog in Ha Noi city area observed under scanning electron microscope. Vet. Sci. Tech. 23, 43–48 (In Vietnamese).

Inthapanya, T., Murrell, K.D., Nongnuch, P., Channan, C., Kuong, K., Tharin, S., et al., 2015. A survey for potentially zoonotic gastrointestinal parasites of dogs and pigs in Cambodia. Acta Parasitol. 60, 601–604.

Jacobi, D.E., Zhu, X., Gasser, R., Chilton, N.B., 1997. PCR-based methods for identification of potentially zoonotic ascaridid parasites of the dog, fox and cat. Acta Trop. 68, 191–200.

Jean, H.K., Park, H., Lee, D., Choe, S., Kim, K.H., Huh, S., et al., 2015. Human infections with Spirometra decipiens pleocercoids identified by morphologic and genetic analyses in Korea. Korean J. Parasitol. 53, 299–305.

Khalil, L.F., Jones, A., Bray, R.A., 1994. Keys to the cestode parasites of vertebrates. CAB International, Wallingford.

Khan, W., Nisa, N.N., Ullah, S., Ahmad, S., Mehmood, S.A., Khan, M., et al., 2020. Epidemiological and genetic data supporting the transmission of Ancylostoma ceylanicum among human and domestic animals. PLoS Negl. Trop. Dis. 6, e1522.

Khan, W., Nisa, N.N., Ullah, S., Ahmad, S., Mehmood, S.A., Khan, M., et al., 2020. Spirometra erinaceieuropaei: The neglected zoonotic parasite of community dogs in Thailand and its genetic diversity among Asian countries. Animals 10, 2154.

Kladkempetch, D., Sahatchai, T., Saruda, T., 2020. Ancylostoma ceylanicum: The neglected zoonotic parasite of community dogs in Thailand and its genetic diversity among Asian countries. Animals 10, 2154.

Le, A.T., Do, L.T., Nguyen, H.T., Do, A.N., 2017. Case report: The first case of human infection by adult of Spirometra erinaceieuropaei in Vietnam. BMC Infect. Dis. 17, 669.

Lieth, B.K., Hieu, D.D., Yen, N.T.H., Do, B.T.A., Lieth, N.V., Anh, L.T.L., 2018. Prevalence of zoonotic hookworm (Ancylostoma spp.) in domestic dogs in Ha Noi City and Phu Tho Province, and pathological characteristics of infected dogs. Vet. Sci. Tech. 25, 49–52 (In Vietnamese).

Lieth, B.K., Huong, N.T., Hieu, D.D., Linh, N.V., Nghiem, N.T., Anh, L.L., et al., 2021. Ancylostoma ceylanicum infections in humans in Vietnam. Parasitol. Int. 84, 102405. https://doi.org/10.1016/j.parint.2021.102405.

Mabdy, M.A.K., Yvonne, A.L.L., Romano, N., Siti, F.M., Seow, H.C., Nan, J.Y., et al., 2012. Prevalence and zoonotic potential of canine hookworms in Malaysia. Parasit. Vectors 5, 88.

Martin, S.W., Mein, A.H., Willeberg, P., 1987. Veterinary epidemiology: Principles and methods. Iowa State University Press, Ames.

Morgan, B.B., Hawkins, P.A., 1949. Veterinary helminthology. Burgess Publishing Company, Minneapolis, Minnesota.

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. Parasit. Vectors 8, 401.

Ngui, R., Yvonne, A.L.L., Rebecca, T., Rohela, M., Mohd, S.M., 2012. Epidemiological and genetic studies on the transmission of Ancylostoma ceylanicum among human and domestic animals. Korean J. Parasitol. 53, 299–305.

Nguyen, T.L., Nguyen, T.P., Johansen, M.V., Murrell, K., Phan, T.V., Dalgaard, A., et al., 2009. Prevalence and risks for fish-borne zoonotic trematode infections in domestic animals in a highly endemic area of North Vietnam. Acta Trop. 112, 198–203.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/.

Rodriguez-Hidalgo, R., Geyser, D., Benitez-Ortiz, W., Geerts, S., Brandl, J., 2002. Comparison of conventional techniques to differentiate between Taenia solium and Taenia saginata and an improved polymerase chain reaction restriction fragment length polymorphism assay using a mitochondrial 12S rRNA fragment. J. Parasitol. 88, 1007–1011.

Thuy, N.H., 1996. Occurrence of gastro-intestinal helminths in dogs in Hanoi and some characteristics of Spirocerca lupi. PhD thesis. National Institute of Veterinary Medicine Research, Hanoi, Vietnam.

Traub, R.J., Robertson, L.D., Irwin, P., Mencke, N., Thompson, R.C.A., 2004. Application of a species-specific PCR-RFLP to identify Ancylostoma eggs directly from canine faeces. Vet. Parasitol. 123, 245–255.

Traub, R.J., Tawin, I., Chantira, S., Yovovalerk, S., Thompson, R.C.A., 2008. PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by Ancylostoma ceylanicum in temple communities in Bangkok. Vet. Parasitol. 155, 67–73.

Tuyen, N.V., 2012. Techniques for raising meat dogs. Thanh Nien Publisher, Hanoi, Vietnam (In Vietnamese).