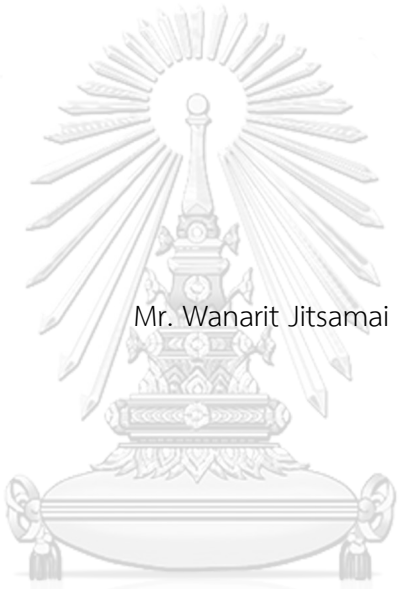
PREVALENCE OF ENTERIC HELMINTHS AND PROTOZOA AND IDENTIFICATION OF HOOKWORM, THREADWORM AND *GIARDIA* SPP. IN CATS IN BANGKOK AND VICINITY, THAILAND

Mr. Wanarit Jitsamai



A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Veterinary Pathobiology
Department of Veterinary Pathology
FACULTY OF VETERINARY SCIENCE
Chulalongkorn University
Academic Year 2019

ความชุกของพยาธิและโปรโตซัวในทางเดินอาหารและการระบุชนิดของพยาธิปากขอ พยาธิเส้นด้าย และเชื้อจีอาร์เดีย ในแมวในกรุงเทพฯ และปริมณฑล ประเทศไทย

นายวนฤทธิ์ จิตสมัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาพยาธิชีววิทยาทางสัตวแพทย์ ภาควิชาพยาธิวิทยา
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	PREVALENCE OF ENTERIC HELMINTHS AND PROTOZOA AND IDENTIFICATION OF HOOKWORM, THREADWORM AND *GIARDIA* SPP. IN CATS IN BANGKOK AND VICINITY, THAILAND
By	Mr. Wanarit Jitsamai
Field of Study	Veterinary Pathobiology
Thesis Advisor	Assistant Professor Dr. WORAPORN SUKHUMAVASI

Accepted by the FACULTY OF VETERINARY SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the FACULTY OF VETERINARY SCIENCE
(Professor Dr. ROONGROJE THANAWONGNUWECH)

DISSERTATION COMMITTEE

..... Chairman
(Associate Professor Dr. THEERAYUTH KAEWAMATAWONG)

..... Thesis Advisor
(Assistant Professor Dr. WORAPORN SUKHUMAVASI)

..... Examiner
(Associate Professor Dr. GUNNAPORN SURIYAPHOL)

..... Examiner
(Associate Professor Dr. SOMPORN TECHANGAMSUWAN)

..... External Examiner
(Assistant Professor Dr. Tawin Inpankaew)

วนฤทธิ์ จิตสมัย : ความชุกของพยาธิและโปรโตซัวในทางเดินอาหารและการระบุชนิดของพยาธิปากขอ พยาธิเส้นด้าย และเชื้อจีอาร์เดีย ในแมวในกรุงเทพฯ และปริมณฑล ประเทศไทย. (PREVALENCE OF ENTERIC HELMINTHS AND PROTOZOA AND IDENTIFICATION OF HOOKWORM, THREADWORM AND *GIARDIA* SPP. IN CATS IN BANGKOK AND VICINITY, THAILAND) อ.ที่ปรึกษาหลัก : ผศ. ดร.วรพร สุขุมาวาสี

การศึกษาในครั้งนี้ ได้ทำการเก็บตัวอย่างมูลแมว จำนวน 835 ตัวอย่าง จากแมวที่มีเจ้าของและแมวที่อาศัยอยู่รอบชุมชน ในระหว่างปี พ.ศ. 2557 – 2559 นำมาตรวจโดยกล้องจุลทรรศน์ ด้วยเทคนิคการป้ายมูลโดยตรง เทคนิคทำให้ไข่ปรสิตจมโดยการปั่นเหวี่ยงโดยใช้สารละลาย PBS และ ethyl acetate เทคนิคทำให้ไข่ปรสิตลอยตัวด้วยวิธีการปั่นเหวี่ยงโดยใช้สารละลายซิงค์ซัลเฟต ผลการตรวจพบความชุกของพยาธิปากขอ มากที่สุดถึงร้อยละ 28.5 ตามด้วยพยาธิไส้เดือนร้อยละ 6.7 เชื้อบิดร้อยละ 5.3 พยาธิใบไม้ในท่อน้ำดีร้อยละ 4.4 พยาธิตืดแมวร้อยละ 2.5 พยาธิเส้นด้ายร้อยละ 1.7 พยาธิตืดปลาร้อยละ 1.4 พยาธิตืดหมัดร้อยละ 1.3 พยาธิเส้นผมร้อยละ 0.1 และ พยาธิใบไม้ในตับร้อยละ 0.1 จากการตรวจหาการติดเชื้อไวรัส FeLV และ FIV พบว่าแมวร้อยละ 7.1 (19/269) และร้อยละ 5.2 (14/269) ให้ผลบวกต่อเชื้อไวรัส FeLV และ FIV ตามลำดับ โดยไม่พบความสัมพันธ์กับการติดเชื้อปรสิตในทางเดินอาหาร นอกจากนี้ จากการตรวจหาโปรตีนของเชื้อจีอาร์เดียร์ด้วยชุดทดสอบ พบว่าร้อยละ 3.9 (9/233) ให้ผลบวก การวิเคราะห์การถดถอยโลจิสติคแบบหลายตัวแปร พบว่า ความสามารถในการออกนอกบ้านและการพบพยาธิปล้องสุกหรือตัวเต็มวัยในมูล เป็นปัจจัยเสี่ยงต่อการติดพยาธิปากขอ ตัวอย่างที่ตรวจพบไข่พยาธิปากขอ จำนวน 207 ตัวอย่าง ได้ตรวจเพิ่มเติมด้วยวิธีปฏิกิริยาลูกโซ่โพลิเมอเรส โดยเพิ่มจำนวนจีน *ITS1, 5.8S* และ *ITS2* พบว่าให้ผลบวก ร้อยละ 59.9 (124/207) และผลการวิเคราะห์ลำดับเบสจำนวน 64 ตัวอย่าง พบว่าเป็นพยาธิปากขอชนิด *Ancylostoma ceylanicum* 63 ตัวอย่าง และชนิด *A. tubaeforme* 1 ตัวอย่าง จากการตรวจเพิ่มเติมด้วยจีน *COX1* พบว่า พยาธิปากขอชนิด *A. ceylanicum* จัดอยู่คนละกลุ่มกับที่พบในมนุษย์ นอกจากนี้ตัวอย่าง DNA ที่สกัดได้จากมูลแมวจำนวนทั้งสิ้น 304 ตัวอย่าง ได้นำมารวมกันเป็นกลุ่มกลุ่มละ 4 ตัวอย่าง จากนั้นตรวจหาสารพันธุกรรมของเชื้อจีอาร์เดียร์ ด้วยวิธีปฏิกิริยาลูกโซ่โพลิเมอเรส โดยเพิ่มจำนวนจีน *SSU rRNA* พบว่า มีเพียง 1 ตัวอย่างที่ให้ผลบวก และตัวอย่างดังกล่าวจัดอยู่ในกลุ่ม assemblage D พยาธิเส้นด้ายได้รับการวินิจฉัยเพิ่มเติมว่าเป็นชนิด *Strongyloides felis* โดยพบลักษณะการคอดเข้ามาของลำตัวบริเวณท้ายรูเปิดช่องคลอด การศึกษาภายใต้กล้องจุลทรรศน์อิเล็กตรอน พบว่า รูปร่างของปากมีลักษณะช่องเปิดเป็นหกเหลี่ยมและถูกล้อมรอบด้วยขอบปากที่ยกนูนขึ้นมาโดยรอบ ทั้งในตัวเต็มวัยเพศผู้และเพศเมีย จากการตรวจด้วยวิธีปฏิกิริยาลูกโซ่โพลิเมอเรส โดยเพิ่มจำนวนจีน *18s rRNA* ร่วมกับการวิเคราะห์ลำดับเบสส่วน hypervariable region I พบว่า มีลำดับเบสที่แตกต่างจากพยาธิเส้นด้ายตัวอื่นในฐานข้อมูล แต่ยังคงอยู่ในกลุ่มของพยาธิเส้นด้าย*ชนิด S. stercoralis* และ *S. procyonis*

สาขาวิชา	พยาธิชีววิทยาทางสัตวแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5775511831 : MAJOR VETERINARY PATHOBIOLOGY

KEYWORD: Ancylostoma spp., Giardia spp., Strongyloides spp., Prevalence, Bangkok, Nonthaburi, Cats, Thailand

Wanarit Jitsamai : PREVALENCE OF ENTERIC HELMINTHS AND PROTOZOA AND IDENTIFICATION OF HOOKWORM, THREADWORM AND *GIARDIA* SPP. IN CATS IN BANGKOK AND VICINITY, THAILAND. Advisor: Asst. Prof. Dr. WORAPORN SUKHUMAVASI

A total of 835 fecal samples collected from client-owned and community cats between 2014 and 2016 were subjected to wet fecal smear and/or PBS-ethyl acetate and/or ZnSO4 centrifugal flotation. *Ancylostoma* spp. was the most common, 28.5%, followed by 6.7% *Toxocara* spp., 5.3% *Cystoisospora* spp., 4.4% *Platynosomum fastosum*, 2.5% *Taenia taeniaeformis*, 1.7% *Strongyloides* spp., 1.4% *Spirometra* spp., 1.3% *Dipylidium caninum*, 0.1% *Eucoleus aerophilus* and 0.1% *Opisthorchis*-like trematode egg. For retroviruses, FeLV and FIV were positive 7.1% (19/269) and 5.2% (14/269), respectively, without association with endoparasitic infection. Based on *Giardia* copro-antigen detection test, 3.9% (9/233) of tested cats were positive. From multivariable logistic regression, ability to access outdoors and having segment or adult worm in feces were significantly associated with *Ancylostoma* spp. infection. From a total of 207 hookworm positive sediment samples were subjected to PCR amplifying *ITS1, 5.8S* and partial *ITS2* regions, 59.9% (124/207) was positive and submitted to sequencing. Out of 64 sequences obtained, 98.4% (63/64) were identified as *A. ceylanicum* and 1.6% (1/64) was found as *A. tubaeforme*. *A. ceylanicum*-positive samples were selected to amplify *COX1* gene and the result suggested that *A. ceylanicum* in this study was most likely have a low potential in zoonotic transmission. For Giardia molecular identification, a total of 304 DNA samples was grouped in a pool of 4 samples and were tested with nested PCR targeting *SSU rRNA* gene of Giardia. Only 1 sample was positive and Giardia assemblage D was confirmed. *Strongyloides felis* was identified base on distinct post vulva constriction. Ultrastructure of *en face* views revealed hexagonal stoma surrounded by circumorally elevations in both male and female free-living adult. Characterization of partial *18s rRNA* including hypervariable region I demonstrated cat threadworm was molecularly distinguishable from other species but still grouped in *S. stercoralis* and *S. procyonis* clade.

Field of Study:	Veterinary Pathobiology	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....

ACKNOWLEDGEMENTS

This research was funded by The 90th Anniversary of Chulalongkorn University fund (Ratchadaphiseksomphot Endowment Fund), The Chulalongkorn University Research Unit for Feline Infectious Disease and Health for Excellence, The Chulalongkorn University Research Unit for Microbial Food Safety and Antimicrobial Resistance, The Chulalongkorn University Research Unit (GRU 6203331007-1). Also, I would like to thank The 100th Anniversary Chulalongkorn University for Doctoral Scholarship for financial support and ERASMUS+ international credit mobility for doctoral program for oversea research financial support.

I would like to express my sincere gratitude and appreciation to my major advisor Asst. Prof. Dr. Woraporn Sukhumavasi for her patience, professional guidance, immense knowledge, kind support and most of all the encouragement to help me get through everything in my Ph.D. work. I am grateful to Assoc. Prof. Dr. Theerayuth Kaewamatawong, Assoc. Prof. Dr. Gunnaporn Suriyaphol, Assoc. Prof. Dr. Somporn Techangamsuwan and Asst. Prof. Dr. Tawin Inpankaew as my thesis committee and given me the valuable comments. I wish to express my deepest gratitude to Prof. Dr. Dwight Bowman and Dr. Ramaswamy Chandrashekar for valuable support and comments. My sincere thanks also go to Prof. Dr. Laura del Rio Alonso for her kind support during my overseas research in University of Murcia, Spain. Also, I would like to thank Dr. Vachira Hunprasit for statistical guidance. My appreciation to Assoc. Prof. Dr. Poom Adisakwattana for his guidance is also recognized. I would like to gratefully thank Prof. Dr. Chollada Buranakarl and the Department of Livestock Development for the opportunity to join the public veterinary services.

I am always thankful the undergraduate students Thitaporn Punyahathaikul, Siriporn Sukkasemsuwan, Rotsakorn Rangsiyanan, Nattawut Phanpongpipat, Latticha Pluemhathaikij, Panumas Harncharoenkul, Tossaporn Powjinda and Pornvipa Pornlumfah for helping in sample collection and laboratory diagnosis. I would like to thank Mr. Nutpiphat Khrutkham for technical support. In addition, I would like to acknowledge all member and all graduate students of Parasitology Unit, Faculty of Veterinary Science, Chulalongkorn University and School of Veterinary Science, University of Murcia, Spain for support and encouragement.

Most of all, I would like to express my special appreciation and thank to my beloved mother (Mrs. Wanida Jitsamai) and my beloved brother (Navarit Jitsamai) for all encouragement and support. Last but not least, I would like to thank my best friends Dr. Alongkorn Kurilung, Pimsiri Piromkij, Kanchanok Iam-aksorn, Korrakod Pramsopee, Pimnipha Siriwattanakul, Chaitawat Sirisereewan, Nuanpan Choosuaingam and Nawaphat Somboonpoonpol for their supports as well as my senior sister graduates Mrs. Juntra Wattanamethanont and Dr. Jitra Sanisuriwong for guidance. Finally, I would like to thank all participated cats and their owners for valuable information.

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Chapter 1

Importance and Rationale

Stray dogs and cats are common in Thailand thus creating persistent nationwide problems especially in urban communities. In Bangkok, Department of Livestock Development revealed that 414,306 cats were brought to participate in rabies control program and a quarter of them were stray cats (dcontrol.dld.go.th). However, the exact number of both stray and owned cat population at the moment remains unknown. It is not uncommon to see stray cats roaming in public area including communities and Thai temples where people usually feed them. In the tropical country like Thailand, queens are prolific species since they can mate and farrow up to 4 litters per year (Faya et al., 2011). If these cats are taken care by unidentified and uncommitted owners or by the owners with irresponsible pet ownership, cats would probably only be fed without receiving other wellness care including vaccination, deworming and neutering. So the population of stray cat could become increasingly and rapidly uncontrolled with possible clinical condition. Since stray cats can roam, urinate and defecate freely, not only do they can pollute the area with their malodorous excreta but they also may harbor important zoonotic disease causing agents apart from rabies.

The tendency of cats to become popular as pets has been increasing. The common living lifestyles of cats are composed of owned and stray. However, although they are owned, they might be raised by having access to outdoors freely unlike strictly indoor cats. Both stray and owned cats can hunt small animals for prey including rodents, cockroaches, amphibians, reptiles, birds, fish, arthropods and snails. Being a natural hunter may increase risks to expose the infective stages of enteric parasites including cyst, metacercaria and plerocercoid. Apart from indirect transmission through consumption of infected intermediate and/or paratenic host, close contact with other cats associated with high density cat housing may lead to protozoal infection including trichomads. Furthermore, they may acquire coccidian oocysts, *Giardia* cyst, *Cryptosporidium* oocysts and soil-transmitted helminths by ingesting infective stages contaminated in the environment. With close interaction with owner, cats can play important role as zoonotic disease reservoirs to human

(Stull et al., 2015). Several enteric parasites in cats are recognized as zoonotic pathogens including soil-transmitted helminths (*Ancylostoma* spp. and *Toxocara cati*), fish- and amphibian-borne Diphyllobothriidean tapeworms (*Diphyllobotrium latum* and *Spirometra* spp.), Cyclophyllidean tapeworms (*Dipylidium caninum*), *Opisthorchis viverrini*, *Toxoplasma gondii* and water-borne protozoa (*Giardia* spp. and *Cryptosporidium* spp.)

In Thailand, several studies reported the prevalence of enteric parasitic infections in cats in Bangkok, Khon Kaen, Pathum Thani, Nakhon Pathom, Nakhon Nayok and Kanchana Buri since 2005 to 2015 (Jittapalapong et al., 2007a; Muksombat et al., 2008; Enes et al., 2010; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014; Rojekittikhun et al., 2015). From these studies, hookworm was almost always the most prevalent enteric parasites detected in cats. *Ancylostoma caninum* and *A. ceylanicum* were recently considered as a neglected zoonotic helminth causing diseases in human including cutaneous larva migrans (CLM) (Tremblay et al., 2000) and eosinophilic enteritis (Bowman et al., 2010). The pathogenesis of this disease is due to the third stage larvae of *Ancylostoma ceylanicum* migrating to and becoming adults in human small intestine (Bowman et al., 2010). Apart from hookworm, there are other feline helminthic infections associated with zoonotic diseases. *Strongyloides* spp. infections in cats were previously reported as uncommon findings (Rojekittikhun et al., 2014; Pumidonming et al., 2017) but these might be underestimated due to the low sensitivity of the fecal examination techniques. Also, the species of *Strongyloides* infecting in cats was not further determined if it is zoonotic. A small liver fluke, *Opisthorchis viverrini,* infection is a common causative agent to induce cholangiocarcinoma, in human living in the northeastern part of Thailand, in which lethal infection can be pursued (Kuper et al., 2000; Khurana et al., 2005). For zoonotic protozoa, while the majority of *Giardia* assemblage in stray dog living in Thai temples was documented as assemblage A (Inpankaew et al., 2007), assemblage of this protozoan in cats has not yet been explored. *Giardia* assemblage A and B have a wide-range host including human, dogs and cats so they are responsible for diarrheic illness in human. Another disease that cats play crucial role for epidemiological aspect is toxoplasmosis. People with immunocompromised

status and naïve pregnant woman are susceptible to infection with *Toxoplasma gondii* as the consequence of reactivated toxoplasmic encephalitis and congenital toxoplasmosis, respectively (Elmore et al., 2010).

Apart from zoonotic enteric parasites, cats can succumb to other important endoparasitic infections. *Platynosomum fastosum*, a small liver fluke of cats living in bile ducts, results in biliary tract obstruction. This liver fluke is also associated with feline cholangiocarcinoma (Basu and Charles, 2014). *Taenia taeniaeformis* can cause intestinal impaction in case of heavy worm burden (Little and Marrinson, 2014) *Tritrichomonus foetus* is responsible for chronic or intermittent large bowel diarrhea (Tolbert and Gookin, 2009). Apart from the intestinal parasites, cats can be infected with respiratory helminths releasing their eggs in feces. Several species of lungworm in cats are identified and related to chronic respiratory diseases (Traversa and Di Cesare, 2013). These parasites may be underdiagnosed due to using inappropriate detection techniques or insufficient mass of fresh feces.

Enteric parasitic infection can be subclinical and neglected by cat owners and veterinarians. This may be partly contributed from tendency of outdoor cats to defecate outside the house and limited mass of feces collected from a thermometer during veterinary physical examination. Direct fecal sample collection from cats under anesthetization with non-invasive technique would allow us to have sufficient mass of fresh feces for more accurate diagnosis. Appropriate selection of diagnostic techniques for fecal samples are important for reveal role of cats as reservoirs for enteric parasitic diseases. Conventional microscopic techniques combined with DNA-based techniques result in high sensitivity of parasite detection especially at the species level. Polymerase chain reaction can be used for species identification of hookworms as well as *Giardia* assemblages. So, the prevalence of zoonotic hookworms, *A. ceylanicum* and *Giardia* assemblage A and B should be determined in order to evaluate role of cats as reservoirs for potentially zoonotic enteric parasites.

From a decade ago, the latest prevalence study of stray cats in Bangkok was published, so current information of enteric parasitic infection in cats in Bangkok and vicinity is in need to be determined. Also, according to One Health concept, role of

cats as reservoirs for potentially zoonotic enteric helminths and protozoa can be addressed.

Objectives of the study

To determine the prevalence of enteric helminths and protozoa from cats in Bangkok and vicinity, Thailand, 2014-2016

To determine risk factors associated with *Ancylostoma* spp. infection in cats in Bangkok and vicinity, Thailand

To reveal the species of *Ancylostoma* spp., *Strongyloides* spp. and *Giardia* spp. infecting cats in Bangkok and vicinity using morphological and molecular identification

Hypothesis

The most common of enteric parasites in cats would still be hookworm and roundworm thus making cats play an important role as reservoirs for potentially zoonotic diseases. The prevalence of enteric parasitic infection in owned cats but having access to outdoors would be not much different from stray cats. This study should be able to reveal both emerging or re-emerging parasites and neglected tropical enteric parasitic infections in cats in Bangkok and vicinity, Thailand.

Literature reviews

Enteric parasites in cats

Enteric parasites in cats can cause various clinical manifestations depending on species of parasites, dose, immunity, ages and co-infection with other pathogens. Enteric parasites in cats are composed of 4 major groups. Firstly, nematodes include hookworms (*Ancylostoma* spp.), roundworms (*Toxocara cati*, *Toxocara malaysiensis*, *Toxascaris leonina*), threadworms (*Strongyloides* spp.), lungworms (*Aelurostrongylus abstrusus, Troglostrongylus brevior*, *Eucoleus aerophilus*), spirurids (*Physaloptera* spp., *Spirocerca* spp., *Ollalunus* spp. and *Gnathostoma* spp.) Secondly, trematodes or flukes includes small liver flukes (*Opisthorchis* spp. and *Platynosomum fastosum*). Thirdly, cestodes or tapeworms includes flea tapeworm (*Dipylidium caninum*), fish-

and amphibian-borne Diphyllobothriidean (*Spirometra* spp. and *Diphylobothrium* spp.), *Joyeuxiella* spp. and *Taenia taeniaeformis*. For the last group, pathogenic protozoa include coccidia (*Cystoisospora felis, C. rivolta*), *Toxoplasma gondii, Giardia* spp., *Cryptosporidium* spp., *Sarcocystis* spp. and trichomonads *(Tritrichomonas foetus, T. blagburni)* (Bowman et al., 2008). Previous study in Bangkok reported that a quarter and one fifth of cats visiting hospitals were seropositive for feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), respectively, (Sukhumavasi et al., 2012) which may lead to opportunistic infections including enteric parasites.

Soil-transmitted feline helminths and their zoonotic importance

Human and animals are infected with soil-transmitted helminths by ingesting developed egg-contaminated soil. World Health Organization (WHO) reported that approximately 1.5 billion people worldwide were suffered from soil-transmitted helminths. Human roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworm (*Necator americanus* and *Ancylostoma duodenale*) are responsible for majority of soil-transmitted helminth infection in human. In addition, 30-100 million human infected with human threadworm, *Strongyloides stercoralis* and 2-80% of children were suffered from infection with dog and cat roundworms, *Toxocara canis* and *Toxocara cati* (Bethony et al., 2006).

In Thailand, hookworm is the most predominant enteric parasites found in cats (Jittapalapong et al., 2007a; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014). Cats are considered as a definitive host for various species of hookworm including *Ancylostoma tubaeforme, A. braziliense, A. ceylanicum* and *Uncinaria stenocephala* (Bowman et al., 2010). Two species of hookworms, *A. caninum* and *A. ceylanicum* were reported in cats in Thailand (Setasuban et al., 1976; Pumidonming et al., 2017), whereas 92% of hookworm-infected cats in Thailand were infected by *A. ceylanicum*, recognized as a re-emerging nematode (Setasuban et al., 1976; Traub, 2013). Recent study also revealed *A. ceylanicum* infection in human from Ubonratchathani province (Niamnuy et al., 2016).

For the biology of hookworm, mating of adult hookworm occurs in small intestine then female worms release fertilized eggs and pass them with feces. Larvae

hatch and develop into a filariform larva, the third stage larva or infective stage, in the suitable condition. Cats are able to be infected by eating infective larvae and/or infected paratenic hosts including rodents and cockroaches apart from direct skin penetration of this larvae. Adult hookworm can cause several clinical manifestations such as acute and chronic blood loss mainly caused by *A. caninum, A. tubaeforme* and *A. ceylanicum*. Human can acquire these parasites by penetration of infective larvae through skin and leave a tract-like lesion called cutaneous larva migrans (CLM) or creeping eruption, in which it is caused by inflammatory responses making patients intensely pruritic. Larvae can be found trapped in the epidermis of tract-like lesion and die within 5-6 weeks (Tremblay et al., 2000). CLM in human can be found in tourists associated with previous history of travelling to Thailand (Caumes et al., 2002; Nakamura-Uchiyama et al., 2002; Miesen, 2003; Malvy et al., 2006; Morsy et al., 2007; van Nispen tot Pannerden et al., 2007; Feldmeier and Schuster, 2012; Veraldi et al., 2013; Creamer, 2014). Moreover, *A. ceylanicum* can penetrate the skin and migrate to small intestine to develop into adults in human (Prociv, 1997).

Toxocara cati is a common enteric parasite in cats following hookworm in Thailand (Jittapalapong et al., 2007a; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014). Similar to hookworm, this parasite is considered as a soil-borne parasite in which transmission occurs via exposure of contaminated-soil. Adult roundworm can release 240,000 eggs per day which tolerate in environment and survive in soil for several years (Cross, 1996). Transmissions occur in 3 ways including comsumption of embryonated eggs and/or infected paratenic hosts and transmammary route (Bowman et al., 2008; Overgaauw and van Knapen, 2013). Human can acquire this parasite by consuming embryonated egg-contaminated food but *Toxocara* infected-human mostly does not develop any clinical signs. However, some cases may have severe illness due to larva migration also called visceral larva migrans (VLM) which damage nervous system and eyes leading blindness (Vamilton et al., 2011).

Threadworm, *Strongyloides* spp., is a soil-transmitted parasitic nematode infecting a wide range of domestic animal species including human worldwide. Cats can be experimentally infected by *Strongyloides stercoralis*, a zoonotic species, and naturally infected by *S. tumefaciens* (reported in North America and India), *S.*

planiceps (reported in Japan and Malaysia) and *S. felis* (reported in India and Australia) (Thamsborg et al., 2017). Dogs have roles in zoonotic transmission cycle of *S. stercoralis* but, in cats, no evidence has been described. Major routes of transmission of *S. stercoralis* are percutaneous and mucosal penetration via oral route whereas the mode of infection for other species in cats is not completely known. Most of cat threadworms reside in the small intestine except for *S. tumefaciens* that inhabits the large intestine nodules. Parasitic females produce larva stage 1 (L1) or partially embryonated eggs, only for *S. planiceps,* and shed in feces. Natural infection with *S. felis* may result in acute watery diarrhea but the experimental infection revealed that cats did not have any clinical signs with focal granulomas and subpleural inflammatory plaques associated with larval migration. Although most *Strongyloides* infections in cats seem to be asymptomatic but potential zoonotic capacity has not yet been determined. So far, there is only one report that demonstrated the prevalence of threadworm in cats in Thailand (Rojekittikhun et al., 2014).

Respiratory nematodes in cats

There are several species of nematodes inhabiting respiratory tract in which their eggs are found in feces. Although these nematodes are important in causing chronic respiratory diseases in cats, there are a few numbers of studies about these parasites. Hairworms or *Eucoleus aerophilus* (formerly *Capillaria aerophila*) and nematodes in the subfamily Metastrongyloidea inhabit the epithelium of trachea and bronchi. Cats become infected with *Eucoleus aerophilus* by eating contaminated food, water and infected-paratenic host including earthworm. Infected cats present clinical signs including cough, nasal discharge, dyspnea and loss of appetite. Young cats infected with hairworms develop severe clinical disease but cats with light infection may not develop any clinical signs leading them to become reservoir hosts with underdiagnosis. Moreover, there was a report in a person infected with *Eucoleus aerophilus* in Serbia and fox was suspected as a reservoir host (Lalosevic et al., 2013). In Thailand, prevalence of *Capillaria* spp. in stray cats in Bangkok and Khon Kaen province are 0.07-1.47% (Jittapalapong et al., 2007a; Muksombat et al., 2008; Chaisiri

et al., 2012). In addition to hairworms, cats can be infected with *Aelurostrongylus abstrusus* and *Troglostrongylus brevior* by eating infected slug and snail. There are evidences which documented that *T. brevior* can be transmitted to human. Although *A. abstrusus* is not considered as a zoonotic pathogen, but they can cause illness in cats due to adults living in bronchioles, alveolar ducts and alveoli leading to respiratory diseases (Traversa and Di Cesare, 2013).

Cestodes and trematodes in cats

Important trematodes in cats include *Platynosomum fastosum* and *Opisthorchis* spp. Cats are infected with *P. fastosum* by eating metacercaria-containing intermediate host such as small reptile, slug, amphibian and possibly cockroach. Adult flukes are found in gall bladder and small bile duct. Clinical signs in cats are not specific including depression, lethargy, abdominal enlargement, vomiting, diarrhea and jaundice (Basu and Charles, 2014). Interestingly, there are reports demonstrating the relationship between *P. fastosum* infection and liver diseases such as cholangiocarcinoma (Andrade et al., 2012) and cystic liver diseases (Xavier et al., 2007). Chronic inflammation caused by adult *P. fastosum* can completely obstruct bile ducts. Once the diagnosis is made, before infection becoming chronic, via fecal examination using fecal sedimentation, praziquantel is a specific treatment to easily clear the infection. However, false-negative findings can occur in case of chronic biliary obstruction making the eggs undetectable in feces. Later, these cats can succumb to lethal infection and positive at necropsy. In Thailand, co-infection of *P. fastosum* and *Clonorchis sinensis* was found in cat with jaundice and hemorrhagic enteritis by necropsy (Nimsuphan et al., 2001). The prevalence of *P. fastosum* in stray cats in Bangkok, Khon Kaen and refugee cats in Nakhon Nayok are 0.07-4% (Jittapalapong et al., 2007a; Muksombat et al., 2008; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014).

Opisthorchiasis is one of the major public health concerns in endemic area like Thailand. *Opisthorchis viverrini*, a zoonotic fluke, inhabits in intra and extra hepatic bile ducts. Cats and human acquire this fluke by eating metacercaria-containing fresh water cyprinoid fish. *O. viverrini* infection is a cause of human

cholangiocarcinoma which has a high incidence in northeastern of Thailand. In 2015, prevalence of this infection in cats in Khon Kaen, Maha Sarakham and Nong Khai is 7-33.3% (Kaewpitoon et al., 2015). International Agency for Research on Cancer under the WHO classifies *O. viverrini* as group 1 biological carcinogen which cause human cancer since 2009. Furthermore, *O. felineus*, found in cats and shared the same life cycle with *O. viverrini*, and *Clonorchis sinensis* are also classified as group 1 biological carcinogen (Pakharukova and Mordvinov, 2016). However, No evidence of *O. felineus* in cats in Thailand has been reported.

Cat tapeworms found in Thailand belong to the order Cyclophyllidea and Pseudophyllidea. Cyclophyllidean tapeworms include *Taenia taeniaeformis, Dipylidium caninum* and *Joyeuxiella* spp. These cyclophyllidean tapeworm are considered as a non-pathogenic cestode which inhabit small intestine. However, heavy infection with these tapeworms may cause impactions and linear-like foreign bodies. Diffuse chronic catarrhal enteritis was reported from a cat with heavy infection of *Joyeuxiella* spp. (Chungpivat et al., 2004). Cats acquire *T. taeniaeformis*, *Joyeuxiella* spp. and *D. caninum* by eating strobilocercus-containing rat liver, cysticercoid-containing reptile and cysticercoid-containing flea, respectively. In Thailand, the prevalence of *T. taeniaeformis* in stray cats in Bangkok and refugee cats in Nakhon Pathom are 0.07-11.7% (Jittapalapong et al., 2007a; Muksombat et al., 2008; Rojekittikhun et al., 2013) while the prevalence of *D. caninum* in stray cats in Bangkok and refugee cats in Kanchana Buri are 0.1-4% (Jittapalapong et al., 2007a; Rojekittikhun et al., 2015). Moreover, *D. caninum*-infected human cases were reported in newborn, children and organ transplant patient but rarely occurred (Molina et al., 2003; Neira et al., 2008; Szwaja et al., 2011; Taylor and Zitzmann, 2011; Narasimham et al., 2013; Sahin et al., 2015). However, no evidence reveals that *T. taeniaeformis* and *Joyeuxiella* spp. is zoonotic.

Diphyllobothrium latum and *Spirometra* spp. are important Pseudophyllidean tapeworms which considered as zoonotic cestodes. Cats acquire these parasites by eating plerocercoid-containing intermediate hosts including reptile, amphibian, fish and birds. Fish is considered as an important intermediate and paratenic host for *D. latum.* Infected cats present clinical signs such as diarrhea, vomiting and weight loss.

Human acquire these parasites by accidentally eating procercoid-infected copepods in drinking water or undercooked plerocercoid-infected flesh and become second intermediate host or paratenic host. Plerocercoid can penetrate skin and move to the other organ forming mass or cyst which clinical signs depending on parasites location such as nervous sign including seizure and paralysis (Liu et al., 2015). In Thailand, prevalence of *Spirometra* spp. in cats in Khon Kaen and refugee cats in Kanchana Buri are 7-13.2% (Muksombat et al., 2008; Rojekittikhun et al., 2015).

Enteric protozoal infections in cats

Waterborne zoonotic protozoa including *Giardia* spp. and *Cryptosporidium* spp. are transmitted by drinking cyst- and oocyst-contaminated water, respectively. After infection, *Giardia* trophozoite attach mucosal surface of small intestine especially proximal part and disrupt function of intestinal villi resulting in maldigestion, malabsorption and diarrhea. The clinical signs of giardiasis depend on the immune status of the host. *Giardia* spp. is characterized using DNA-based techniques into assemblage A to H. Cats become infected with *Giardia* assemblage A, B and F which have been proposed as *G. duodenalis*, *G. enterica* and *G. cati*, respectively (Thompson and Ash, 2016). Some studies further characterized *Giardia* assemblage A into assemblage A1 and A2 which cats were mainly infected with assemblage A1 and F and human were mainly infected with assemblage A2 and B. In Thailand, previous study revealed that majority of *Giardia* spp. from stray dogs in Thai temples were assemblage A (Inpankaew et al., 2007). However, assemblage of cats in Thailand remains unknown. In stray cats, prevalence of *Giardia* spp. in Bangkok and refugee cats in Nakhon Nayok and Kanchana Buri are 0.3-3% (Rojekittikhun et al., 2014; Rojekittikhun et al., 2015).

Similar to *Giardia*, *Cryptosporidium* spp. can cause diarrhea in cats depending on cat immune status. Cats can become infected with two species of *Cryptosporidium*, *C. felis* and *C. parvum*. The latter species is recognized as opportunistic zoonotic infection causing severe illness in immunocompromised patients. The prevalence of *Cryptosporidium* in cats was reported in stray cats from

Nakhon Nayok, 2.5%, and in cats in which it was identified as a *C. felis* using polymerase chain reaction (Koompapong et al., 2014).

Enteric protozoa commonly found in cats especially kittens are coccidian including *Cystoisospora felis* and *C. rivolta*. Cats acquire by eating sporulated oocyst contaminated in the environment and environmentally resistant or eating monozoic tissue cyst-containing intermediate host such as rodents. *Cystoisospora* spp. Is host-specific and they it infects intestinal epithelium affecting structure of villi and microvilli lead to maldigestion and malabsorbtion. Infected kittens become malnutrition, weight loss and diarrhea (Lappin, 2010). In Thailand, prevalence of *Cystoisospora* spp. in stray cats in Bangkok and refugee cats in Pathum Thani, Nakhon Pathom, Nakhon Nayok and Kanchana Buri are 1-6% (Jittapalapong et al., 2007a; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014; Rojekittikhun et al., 2015).

Unlike monoxenic coccidian, *Toxoplasma gondii* is heteroxenous coccidian in which intermediate host can be part of the life cycle. *T. gondii* is an important protozoan causing severe diseases in human. Cats and many species belonging to family Felidae serve as a definitive host so they can produce *Toxoplasma* oocyst shedding in the environment. However, majority of human toxoplasmosis occurs from consumption of bradyzoite-containing tissue cyst in undercooked meat (Jones and Dubey, 2010). Reactivated toxoplasmosis can lead to encephalitis in human especially in immunocompromised patients including HIV-infected patients, ongoing chemotherapy and organ transplant patients (Jones et al., 1999). *Toxoplasma*-seronegative antibody pregnant woman is susceptible to develop prenatal toxoplasmosis for her fetus leading to negative consequences such as abortion, mental retardation, congenital anomaly and blindness. In USA, human toxoplasmosis results in socio-economic loss more than 3.3 billion USD per years ((Hoffmann et al., 2015). In Bangkok, a previous report showed that 6.4% of cat owners had seropositive for *Toxoplasma* (Sukthana et al., 2003). In contrast to human, postnatal *Toxoplasma* infection in cats generally results in non-clinical disease except for immunocompromised cats for example, feline immunodeficiency virus (FIV) and/or feline leukemia virus (FeLV) infected cats and in kitten. Clinical manifestations in cat include loss of appetite, lethargy, fever, weight loss, pneumonia, respiratory distress,

hepatitis, jaundice and neurological signs. Uncommon clinical signs in cats may occur including lymphadenopathy, vomiting, diarrhea and muscle pain (Elmore et al., 2010). Naïve pregnant queens infected with *T. gondii* may result in abortion and stillbirth. Although cats serve as an important role to produce *Toxoplasma* oocysts, infected cats release large number of oocysts less than 3 weeks, mostly for 1 week. Also, optimal condition of the environment is required for oocyst sporulation for 1-5 days to become infective. Additionally, only 1% of *Toxoplasma*-seropositive cats shed oocyst (Dubey and Jones, 2008). Therefore, cats may not be responsible for a major source of human toxoplasmosis (Jones and Dubey, 2010). In Thailand, prevalence of *Toxoplasma* seropositive in stray cats in Bangkok are 4.8-11% (Sukthana et al., 2003; Jittapalapong et al., 2007b) whereas cats visiting hospital for any service is 10.1% (Sukhumavasi et al., 2012).

Lastly, trichomonad, a mucosoflagellate protozoan, causes non-clinical signs to chronic and intermittent large bowel diarrhea in cats especially in multi-cat household with high density. This flagellate is classified into two species, *Tritrichomonas foetus* and *Tritrichomonas blagburni* (Yao and Koster, 2015). Cats become infected by ingestion of trophozoite-contaminated food and water (Rosypal et al., 2012). Trophozoites, only stage in life cycle, reside in ileum and colon. Also, this parasite was found in uterus of cats with pyometra (Dahlgren et al., 2007).

Parasitological diagnosis of feline enteric parasites

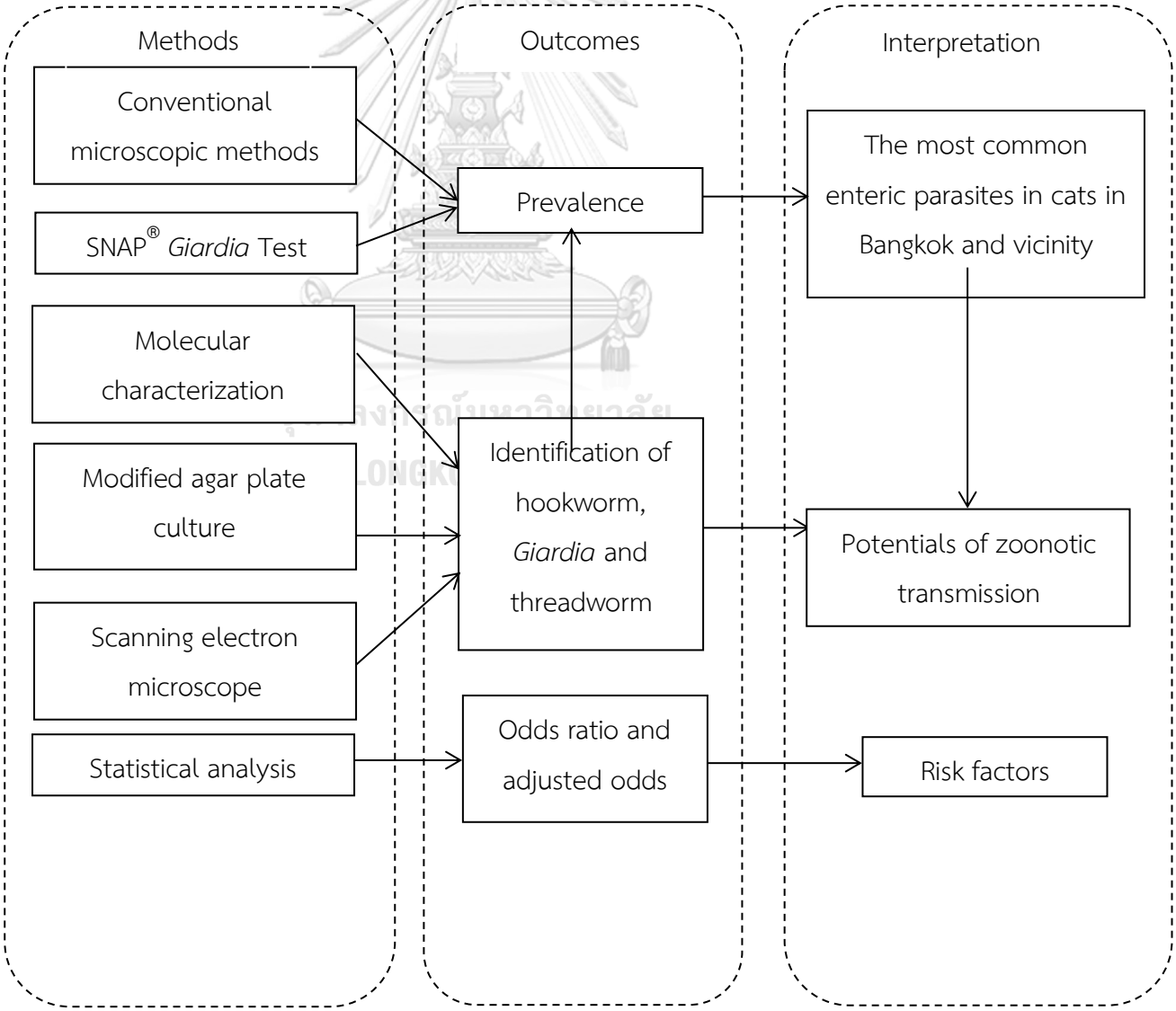
All of diagnostic stage of enteric parasites including eggs, trophozoites, cysts and oocysts can be found in feces. Conventional microscopic methods including wet fecal smear, flotation and sedimentation techniques commonly use in routine work. Wet fecal smear is beneficial to detect motile parasitic stage including trophozoites and nematode larvae in freshly collected feces but its sensitivity is low due to using small mass of feces. While sedimentation techniques can recover almost all parasitic eggs which include operculated eggs, flotation techniques provide higher recovery rate for non-operculated eggs, oocyst and cyst compared to sedimentation. Centrifugation can be applied in order to increase recovery rate of parasitic eggs from flotation technique (Dryden et al., 2005). For flotation technique, specific gravity and

type of flotation solution are important factors to successfully recover parasitic eggs. $ZnSO_4$ with specific gravity 1.18 is the solution of choice to recover *Giardia* cyst as it leaves internal structure of the cyst more intact. For the sedimentation technique, formalin ethyl acetate which adapted from formalin ethyl ether is suggested for non-herbivore feces including human, cats and dogs. Ethyl acetate is able to dissolve and separate organic materials in feces thus making background clearer to observe parasitic eggs under the light microscope. The limitations of microscopic methods are low sensitivity, labor-intensive and only identify morphology at the genus level. Moreover, microscopic technique cannot distinguish *Toxoplasma gondii* oocyst from *Hammondia hammondi* and *Besnoitia* spp. oocysts due to their same size (Jones and Dubey, 2010). There are several methods to increase sensitivity of motile stage detection. Baermann's method is based on the movement of larvae to the bottom of funnel thus this method yields motile larvae prior to use in experimental infection or identification (Thamsborg et al., 2017). Agar plate culture is another method to detect nematode larvae by providing nutrients for environmental stage of nematode such as *Strongyloides* (Koga et al., 1991). For *Tritrichomonas foetus*, culture with Diamond media can increase sensitivity of detection (Tolbert and Gookin, 2009).

Advanced techniques including protein and DNA-based detection techniques provide higher sensitivity compared to conventional microscopic methods (Traub et al., 2008). Several protein-based techniques were designed in order to detect parasite membrane protein or parasite producing protein by using specific antibody including enzyme-linked immunosorbent assay (ELISA), immunochromatography and immunofluorescence method. For DNA-based techniques, highly conserved gene with multiple copies including internal transcribed spacer 1, 2 (ITS1, 2) (Palmer et al., 2007), and small subunit ribosomal DNA (SSU rDNA) (Sulaiman et al., 2003) are commonly amplified in order to identify the parasite at the species level. Besides ITS1, 2 gene, sequence of the cytochrome c oxidase subunit 1 (cox1) are used to differentiate the clades of *Ancylostoma ceylanicum* which can define the zoonotic potential of hookworm in animals by comparing with human clade (Inpankaew et al., 2014a)

For *Strongyloides* species, due to highly conserved regions of small SSU rDNA between *Strongyloides* species, hyper-variable region (HVR) in SSU rDNA are suggested for species identification (Hasegawa et al., 2009). Common targeted genes including small subunit ribosomal DNA (SSU rDNA) and transcribed spacer 1, 2 (ITS1, 2) are used for *Giardia* spp. detection. In addition, the specific genes including triosephosphate isomerase (TPI), *Giardia* spp. specific gene ß-giardin, and the glutamate dehydrogenase (GDH) genes are selected to characterize the assemblage and sub-assemblage level (Thompson and Ash, 2016)

Conceptual framework



Chapter 2

Diversity of zoonotic and neglected enteric parasitic infections and risk factors associated with *Ancylostoma* spp. infection in client-owned and community cats in Bangkok and vicinities, Thailand

Wanarit Jitsamai[a], Nutpiphat Khrutkham[b(former)], Vachira Hunprasit[c], Ramaswamy Chandrashekar[d], Dwight Bowman[e], Woraporn Sukhumavasi[b e*]

[a]Graduate program in Pathobiology, [b]Parasitology Unit, Department of Pathology, [c]Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University Bangkok 10330, Thailand

[d]IDEXX Laboratories, Inc., Westbrook, ME, USA

[e]Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

[e] Feline Infectious Disease and Health for Excellence Research Unit, Microbial Food Safety and Antimicrobial Resistance Research Unit, Animal Vector-Borne Disease Research Unit, Chulalongkorn University

*Corresponding

e-mail address: vetkwan@hotmail.com

Keywords:

Ancylostoma spp., cat, enteric parasites, retrovirus, *Giardia*, Thailand

Abstract

Prevalence study of enteric parasites in cats in Bangkok and vicinities has not been updated in over 13 years despite increased population of cats in this region and availability of endoparasite control products. In order to address this question, 509 fecal samples collected from client-owned and community cats between 2014 and 2015 were subjected to wet fecal smear and/or PBS-ethyl acetate and/or $ZnSO_4$ centrifugal flotation and microscopically examined for the presence of parasitic stages. The results revealed that 32.0% (163/509) of tested samples were positive for any parasite. *Ancylostoma* spp. was the most common, 21.6%, followed by 6.9% *Toxocara* spp., 3.7% *Platynosomum fastosum*, 3.5% *Cystoisospora* spp., 2.9% *Taenia taeniaeformis*, 1.6% *Spirometra* spp., 0.4% *Dipylidium caninum* and 0.2% *Opisthorchis*-like trematode egg. Using the *Giardia* copro-antigen detection test for the subset of samples, 233 samples, 3.9% (9/233) were positive for *Giardia* cyst wall protein. For detection of feline leukemia virus antigen and antibody against feline immunodeficiency virus, 7.1% (19/269) and 5.2% (14/269) of tested cats were seropositive, respectively, whereas no heartworm circulatory antigen was detected. No association between retroviral infections and endoparasite infection was found. From multivariable logistic regression, ability to access outdoors (adjusted OR= 3.22, 95% CI: 1.42 – 7.87) and having segment or adult worm in feces (adjusted OR= 3.31, 95% CI; 1.34 – 8.21) were significantly associated with *Ancylostoma* spp. infection. This work contributes the most updated data of feline enteric parasites prevalence in Bangkok and vicinities in which fecal samples were directly collected from cats. Consequently, this study emphasizes that diagnosis of parasitic infections and the routine use of antiparasitic preventative should be encouraged to the veterinarians and owners in order to reduce the reservoir of zoonotic helminths.

Introduction

Cats tend to be more popular pets in urban society due to less space required for caring. However, if cats are raised outdoors and not neutered, they are very prolific, making cat population control challenging in a large city like Bangkok. Recently, Department of Livestock Development reported that over 400 thousand cats resided in Bangkok (dcontrol.dld.go.th). Not only do they cause problems within the community as a result of improper soiling, their fecal materials can also harbor important infectious zoonotic pathogens including parasites (Conlan et al., 2011; Chen et al., 2012; Stull et al., 2015). High temperatures and humidity contributing to year round tropical conditions in Thailand mean developing stages of parasites can easily become infective in the environment especially for soil-transmitted helminths including *Ancylostoma* spp. and *Toxocara cati* (Overgaauw and van Knapen, 2013; Holland, 2017).

From several provinces of Thailand, the prevalence of enteric parasitic infection in cats was reported between 2005 to 2014 (Muksombat et al., 2008; Enes et al., 2010; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014; Rojekittikhun et al., 2015; Pumidonming et al., 2017) but only one report was from Bangkok in which samples were collected in 2005 (Jittapalapong et al., 2007a). In Southeast Asian countries, prevalence reports were available from 4 countries including Laos, Malaysia, Vietnam, and Indonesia and hookworm was also the most common in these countries.

Among reports in Thailand, hookworm is the most common parasite in almost all studies. Cats are considered a definitive host for various species of hookworm including *Ancylostoma tubaeforme, A. braziliense, A. ceylanicum* and *Uncinaria stenocephala* (Bowman et al., 2010). However, two species of hookworms, *A. caninum* and *A. ceylanicum*, were reported in cats in Thailand (Setasuban et al., 1976; Pumidonming et al., 2017). Cats are infected by eating infective larvae contaminated in the environment and/or infected paratenic hosts including rodents and cockroaches apart from direct skin penetration of larva. Adult hookworm can cause several clinical manifestations such as acute and chronic blood loss, mainly caused by *A. caninum, A. tubaeforme* and *A. ceylanicum*. In human, hookworm

infection can occur via skin penetration of third-stage larvae causing cutaneous larva migration (Bowman et al., 2010). Moreover, *A. ceylanicum* can penetrate the skin and migrate to small intestine to develop into the adult stage in humans (Prociv, 1997).

In Thailand, *Toxocara* spp. is also a common enteric parasite in cats following hookworm (Jittapalapong et al., 2007a; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014). Similar to hookworm, this parasite is considered a soil-transmitted helminth in which transmission occurs three ways: consumption of embryonated eggs from the environment and/or infected paratenic hosts, and via the transmammary route, making Toxocara spp. infection very common in kittens. (Bowman et al., 2008; Overgaauw and van Knapen, 2013).

For trematodes in cats, they are infected by consuming metacercaria-containing intermediate hosts. Proposed life cycle of *Platynosomum fastosum* showed that snail, isopod and lizard serving as intermediate hosts (Basu and Charles, 2014) whereas *Opisthorchis* spp. uses fresh-water cyprinoid fish for development of metacercaria (Kaewpitoon et al., 2015). For pathogenicity, adult *P. fastosum* lives in gall bladder and biliary tracts causing non-specific clinical signs including depression, lethargy, abdominal enlargement, vomiting, diarrhea and jaundice due to biliary obstruction (Basu and Charles, 2014). In northeastern part of Thailand, apart from infection in cats, human infection with *Opisthorchis viverrini* has been found endemic leading to human cholangiocarcinoma. (Muksombat et al., 2008; Enes et al., 2010).

Tapeworm infection in cats are caused by *Taenia taeniaeformis, Dipylidium caninum* and *Spirometra* spp. Although they are considered as nonpathogenic but there were reports on intestinal obstruction, impaction and linear-like foreign bodies caused by heavy infection of *T. taeniaeformis* (Wilcox et al., 2009). Also, *Spirometra* spp. can make animals vitamin B12 deprived hence making them succumbed to anemia (Marchiondo et al., 1989).

For protozoan infections in cats, coccidia and mucosoflagellate including *Cystoisospora felis, C. rivolta*, *Tritrichomonas foetus*, *T. blagburni*, *Giardia* spp. and *Cryptosporidium* spp. are important pathogens. *Giardia* spp., waterborne and potentially zoonotic protozoan, is transmitted by drinking cyst-contaminated water leading to small bowel diarrhea (Thompson and Ash, 2016). Enteric protozoa

commonly found in cats especially kittens causing small bowel diarrhea are coccidian including *Cystoisospora felis* and *C. rivolta*. Consumption of sporulated oocyst contaminated in the environment and fomites or eating monozoic tissue cyst containing rodent intermediate host are mode of coccidian transmission (Lindsay et al., 2014).

Apart from helminth and protozoan infections, retrovirus infections are common in cats in Thailand. Feline leukemia virus and feline immunodeficiency virus were reported using commercial in-house test kits with the prevalence ranged 16.5-24.5% and 5.4-20.1%, respectively (Sukhumavasi et al., 2012; Nedumpun et al., 2015). These viruses can make cats succumbed to immunosuppressive and susceptible to other infections such as viral, bacterial, protozoal, and fungal origin (Hartmann, 2011). However, there was no study conducting to address if there is an association between retroviral infections and endoparasite infections.

Bangkok metropolitan is the crossroad that hosts, parasites and suitable environments meet thus making this area suitable for epidemiological study of enteric parasite diversity in cats in a tropical country. Also, in the era of several therapeutic specific options available to control endoparasites, the prevalence of enteric parasites in cats in Bangkok should be up to date. So recommendation on routine fecal examination and parasite control can be properly encouraged.

Materials and Methods

Study design, sample collection and history taking

The retrospective study was conducted from the data of parasitological diagnostic results of cats brought for mobile veterinary and neutering services in Bangkok and vicinities between the year 2014 and 2015. The researchers were responsible for performing laboratory diagnostic services. A total of 509 fecal samples were collected from cats during post-operation recovery by rectum and/or colon flushing using 10 ml sterile normal saline via sterile disposable feeding tube no. 6 or no. 8 (Bevermed Ind, Thailand) (Tolbert and Gookin, 2009). For a subset of this population, 269 cats, 1 ml heparinized blood samples were obtained from femoral vein using 22 or 23 G 1” needles. Both types of samples were transported in cold

chain manner using ice pack-containing insulated box for further laboratory processing and testing within the same day of sample collection.

Based on the data providing by cat owners on-site or via telephone calls, signalments including sex (male, female), age (≤1 year, 1-5 years, >5 years) and ability to access outdoors (outdoors, strictly indoors) were obtained. Possible factors that could be associated with enteric parasitic infections were recorded using questionnaires based on following history, cat living lifestyle, hunting behavior, ecto- and endoparasite prevention, gastrointestinal clinical signs and retroviral infection status due to FeLV and FIV infections in which they were evaluated in this study.

Fecal examination

Prior to coprological diagnosis, all fecal strainers and glass droppers were thoroughly clean followed by immersing them in boiling water for 10 mins to decontaminate if any. Conventional fecal examination methods including wet fecal smear and/or phosphate-buffered saline (PBS)-ethyl acetate centrifugal sedimentation and/or $ZnSO_4$ centrifugal flotation (specific gravity 1.18) were respectively conducted depending upon availability of remaining samples. Microscopic examination was conducted and confirmed by at least an experienced examiner. All fecal samples were freshly tested using wet fecal smear at sample collection site. The rest of techniques were performed to detect the presence of parasitic stages within the same day upon transporting the samples to the parasitological diagnostic laboratory. Apart from coprological diagnosis by conventional methods, detection of *Giardia* antigen (SNAP® *Giardia* Test, IDEXX Laboratories, USA) was performed with 233 fresh fecal samples. Out of these samples, 13 PBS-containing fecal sediment samples, previously stored in -20ºC before the test arrival, were later tested. To ensure that sediment could be reliably used, sediment from *Giardia* cyst positive control was tested positive by this antigen test.

Blood examination for FeLV/FIV and heartworm infections

To test the cat blood for the presence of FeLV and *Dirofilaria immitis* circulatory antigens and antibody against FIV, plasma samples of 269 cats pertaining completed history were isolated post centrifugation at 9,000 rpm (6,792 xg) for 5 min at room temperature and stored in -20°C until tested with SNAP® Triple Test (IDEXX Laboratories, USA) according to manufacturer's instruction.

Statistical analysis

The data were analyzed using statistical program R (R Core Team, 2019). Demographic data and the prevalence of enteric parasites in cats were demonstrated using descriptive statistics as proportion and 95% confidence interval (95% CI) of proportion. Detection proportions of each parasitological method were compared by proportion Z-test of each enteric parasite. The association between factors and cats infected with *Ancylostoma* spp. was tested. Univariate logistic regression was performed to screen all explanatory variables and expressed in odds ratio (OR), and 95% CI by *Epicalc* V3.5.1.6 (Chongsuvivatwong, 2019) package. The multicollinearity among explanatory variable was evaluated by Chi-square test (p-value < 0.05). In case of multicollinearity among variables, the variable with higher biological plausibility was retained for multivariable analysis. Variables from the univariate analysis with p-value $\leq$ 0.2 and without marked multicollinearity among variables were included in the full multivariable logistic regression for model selection (Dorris et al., 2002). A backward stepwise variable selection procedure was performed based on AIC. All possible 2-way interaction were further tested. The Goodness-of-fit test for final multivariable logistic model was assessed by Hosmer-Lemeshow Goodness-of-fit test.

Results

Demographic data

Based on the location where the cats lived at the time of sample collection, 509 cats were from 4 central provinces of Thailand including Bangkok, Nonthaburi, Pathum Thani and Samut Prakan. For 44.6% (227/509) of this population, completed

signalment and questionnaires were obtained. Signalment and history of cats are demonstrated in Table 1.

Table 1 Demographic data showing signalment and history of cats living in Bangkok and vicinities 2014-2015 (n = 227)

Demographic data	% (n)
Sex	
Male	34.8 (79)
Female	56.4 (128)
Age	
≤ 1 year	24.2 (55)
1-5 years	23.8 (54)
> 5 years	5.3 (12)
Ability to access outdoors	
Outdoors	63.0 (143)
Strictly indoors	36.1 (82)
Multi-cat household	60.8 (138)
Living with dogs in the same household	30.8 (70)
Expose to other cats outside	37.9 (86)
Expose to other dogs outside	29.1 (66)
Monthly routine deworming	9.3 (21)
Monthly ectoparasite prevention	18.5 (42)
Presence of fleas	31.7 (72)
History of gastrointestinal clinical signs	
Diarrhea	25.1 (57)
Vomiting	49.3 (112)
History of segment and/or adult worm presenting in feces	11.9 (27)
Hunting behavior	
Rodents	42.7 (97)
Small reptiles	27.3 (62)
Insects	38.3 (87)
Birds	11.9 (27)
Drinking water from natural sources	32.2 (73)

Prevalence of enteric parasite infections in cats

From a total of 509 fecal samples, 32.0% (163/509) (95% CI; 28.1 - 36.2%) of tested samples were positive for any parasite by conventional coprological and microscopic examination. *Ancylostoma* spp. eggs were found 21.6% (110/509) of all tested samples thus making hookworm the most predominant among endoparasite-infected cats, 67.5% (110/163), in this study. The prevalence of other enteric parasites based on morphological identification of their eggs or oocysts including *Toxocara* spp., *Platynosomum fastosum*, *Cystoisospora* spp., *Taenia taeniaeformis*, *Spirometra* spp., *Dipylidium caninum* and *Opisthorchis*-like trematode were 6.9%, 3.7%, 3.5%, 2.9%, 1.6%, 0.4% and 0.2%, respectively (Table 2). Since the number of tested cats from Pathum Thani and Samut Prakan provinces was limited, 3, 13, respectively, prevalence of each parasite is particularly shown for the cats living in Bangkok and Nonthaburi provinces (Fig. 1). Examined by conventional fecal examination techniques, cats residing in Bangkok and Nonthaburi were positive for any endoparasite 30.4% (103/339) and 33.8% (52/154), respectively. The spatial distribution of each parasite found in cats living in 5 different regions of Bangkok and 6 districts of Nonthaburi provinces is shown based on groups of nematodes, flukes, cestodes, and protozoan (Fig. 2).

Table 2 Prevalence of enteric parasitic infections in cats from Bangkok and vicinities, 2014-2015 (n = 509)

Parasites	% (n)	95% CI
Nematodes		
Ancylostoma spp.	21.6 (110)	18.3 – 25.4
Toxocara spp.	6.9 (35)	5.0 - 9.4
Flukes		
Platynosomum fastosum	3.7 (19)	2.4 - 5.8
***Opisthorchis*-like**	0.2 (1)	0.0 - 1.1
Cestodes		
Taenia taeniaeformis	2.9 (15)	1.8 - 4.8
Spirometra spp.	1.6 (8)	0.8 - 3.1
Dipylidium caninum	0.4 (2)	0.1 - 1.4
Protozoa		
Cystoisospora spp.	3.5 (18)	2.3 - 5.5

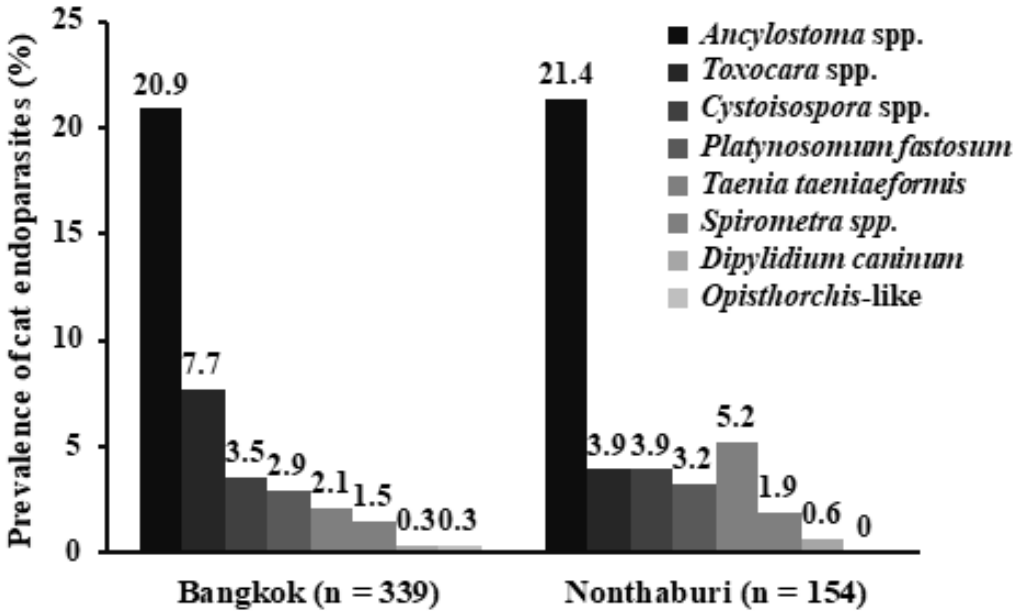


Figure 1 Prevalence of enteric parasitic infections in cats living in Bangkok (n = 339) and Nonthaburi provinces (n = 154), between year 2014 and 2015

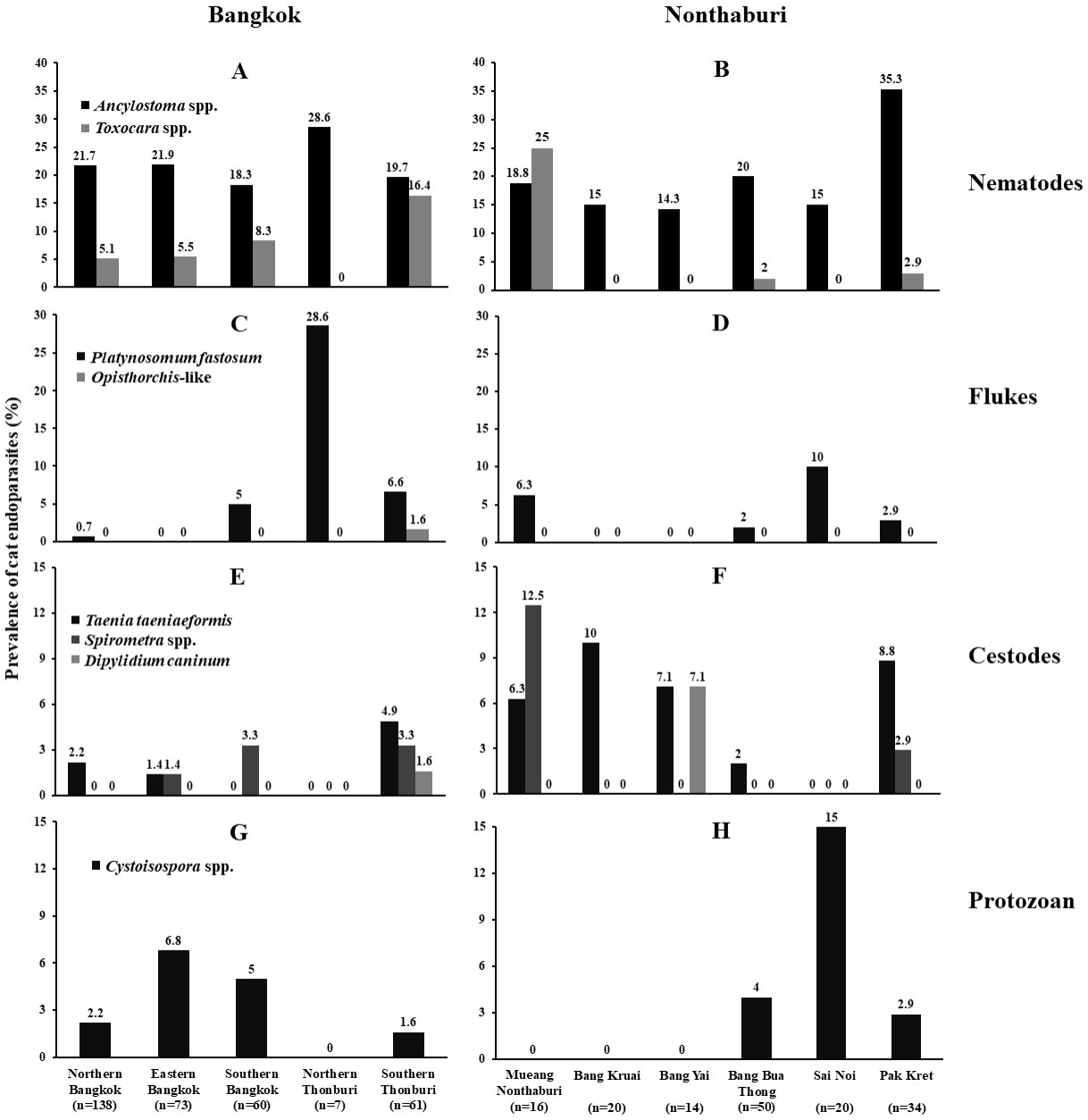


Figure 2 Prevalence of enteric parasitic infections in cats living in 5 different regions Bangkok (A, C, E and G) versus prevalence in cats living in 6 different districts Nonthaburi province (B, D, F and H) between year 2014 and 2015. Distribution of endoparasites was grouped into nematodes (A, B), flukes (C, D), cestodes (E, F) and protozoan (G, H).

In order to compare the recovery rate of different parasites between each technique, a total of 105 samples were examined by all 3 conventional techniques.

Since the mass of fecal sample from each cat was limited, samples were gradually tested based on priority tapering down from wet fecal smear (n=509), PBS-ethyl acetate centrifugal sedimentation (n=229) and $ZnSO_4$ centrifugal flotation (n=105), respectively. The prevalence of parasite infections obtained from each technique are shown in Table 3. Recovery rates of hookworm eggs was higher by using PBS-ethyl acetate centrifugal sedimentation technique and by using $ZnSO_4$ centrifugal flotation than did by wet fecal smear ($p<0.01$). Detection rate of coccidia oocysts was better by using $ZnSO_4$ centrifugal flotation than did wet fecal smear and PBS-ethyl acetate centrifugal technique ($p<0.01$). Interestingly, although PBS-ethyl acetate centrifugal sedimentation technique seemed to be able to detect higher number of liver fluke-infected cats but the difference between sedimentation and flotation technique was not statistically significant. For both tapeworms, any conventional techniques could yield the similar recovery rate of *T. taeniaeformis* and *Spirometra* spp.

Apart from comparative recovery rate of parasite detection, 51.7% (263/509) of this studied population were only examined by wet fecal smear due to limited fecal sample. The prevalence of *Ancylostoma* spp., *Toxocara* spp., *Taenia taeniaeformis*, *Dipylidium caninum*, *Cystoisospora* spp. and *Platynosomum fastosum* were 6.8% (18/263), 2.7% (7/263), 2.7% (7/263), 0.8% (2/263), 0.4 % (1/263) and 0.4% (1/263), respectively. However, none of *Spirometra* spp. egg was detected in this wet fecal smear-only subpopulation.

Table 3 Comparative prevalence of feline endoparasites examined by 3 different coprological examination techniques using the same fecal sample (n=105)

Parasite	Wet fecal smear % (n)	PBS-ethyl acetate centrifugal sedimentation % (n)	$ZnSO_4$ centrifugal flotation % (n)
Ancylostoma spp.	10.5 (11)[a]	36.2 (38)[b]	32.4 (34)[b]
Toxocara spp.	5.7 (6)[a]	10.5 (11)[a]	12.3 (13)[a]
Platynosomum fastosum	0.0 (0)[a]	5.7 (6)[b]	1.0 (1)[a,b]
Cystoisospora spp.	0.0 (0)[a]	2.9 (3)[a]	10.5 (11)[b]
Taenia taeniaeformis	2.9 (3)[a]	0.0 (0)[a]	1.0 (1)[a]
Spirometra spp.	3.8 (4)[a]	2.9 (3)[a]	2.9 (3)[a]

[a,b] represent the significantly difference ($p<0.05$) using proportion Z-test (one-side)

When we analyzed the different types of infections, single infection with *Ancylostoma* spp. was the most predominant, 15.9%, followed by single infection with *Toxocara* spp., 4.1%. For dual infections, *Ancylostoma* spp. and *Cystoisospora* spp. co-infection was the most common, 1.8%. The prevalence of each type of infections, allocated as single, dual and multiple infections, is shown in Table 4.

Table 4 Types of endoparasite infections in cats living in Bangkok and vicinities between 2014-2015 (n = 509)

Type of infections	% (n)	95% CI
Single infection		
Ancylostoma spp.	15.9 (81)	12.7 – 19.1
Toxocara spp.	4.1 (21)	2.4 – 5.8
Cystoisospora spp.	1.1 (6)	0.3 – 2.2
Platynosomum fastosum	1.3 (7)	0.4 – 2.4
Taenia taeniaeformis	1.6 (8)	0.5 – 2.7
Spirometra spp.	0.8 (4)	0.1 – 1.6
Dipylidium caninum	0.2 (1)	0.0 – 0.6
Dual infections		
Ancylostoma spp. and *Cystoisospora* spp.	1.8 (9)	0.7 – 3.0
Ancylostoma spp. and *Platynosomum fastosum*	1.2 (6)	0.3 – 2.2
Ancylostoma spp. and *Toxocara* spp.	1.0 (5)	0.1 – 1.9
Ancylostoma spp. and *Taenia taeniaeformis*	0.4 (2)	0.0 – 1.0
Toxocara spp. and *Cystoisospora* spp.	0.4 (2)	0.0 – 1.0
Toxocara spp. and *Taenia taeniaformis*	0.2 (1)	0.0 – 0.6
Toxocara spp. and *Spirometra* spp.	0.2 (1)	0.0 – 0.6
Platynosomum fastosum and *Spirometra* spp.	0.2 (1)	0.0 – 0.6
Taenia taeniaeformis and *Dipylidium caninum*	0.2 (1)	0.0 – 0.6
Multiple infections		
Ancylostoma spp., *Toxocara* spp. and *Platynosomum fastosum*	0.6 (3)	0.0 – 1.3
Ancylostoma spp., *Cystoisospora* spp. and *Spirometra* spp.	0.2 (1)	0.0 – 0.6
Ancylostoma spp., *Taenia taeniaeformis* and *Spirometra* spp.	0.2 (1)	0.0 – 0.6
Ancylostoma spp., *Toxocara* spp., *Platynosomum fastosum* and *Taenia taeniaeformis*	0.2 (1)	0.0 – 0.6
Ancylostoma spp., *Toxocara* spp., *Platynosomum fastosum, Taenia taeniaeformis,* and *Opisthorchis*-like	0.2 (1)	0.0 – 0.6

Using the SNAP® *Giardia* Test for the subset of samples, 233 samples, *Giardia* cyst wall protein was detected in 3.9% (95% CI; 1.4 – 6.4%) of tested cats (9/233). Among these tested cats, *Giardia* spp. co-infection with *Ancylostoma* spp. was the

most common at 2.6% (95% CI 0.6 - 4.6%) followed by single *Giardia* spp. infection 0.9% (95% CI; 0.0 – 2.1%) and *Giardia* spp. with *Toxocara* spp. and *Cystoisospora* spp. 0.4% (95% CI; 0.0 – 1.2%). For the type of infection, single, dual and multiple infection(s) of different types of endoparasites are demonstrated in Table 4.

Seroprevalence of feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and heartworm (*Dirofilaria immitis*) infection in cats and association with endoparasite infections

A total of 269 blood samples collecting from Bangkok were tested with SNAP®Triple test. Feline leukemia virus (FeLV) antigen and antibody against feline immunodeficiency virus (FIV) were found in 7.1% (19/269) (95% CI; 4.6 - 10.8%) and 5.2% (14/269) (95% CI; 3.1 - 8.5%), respectively, whereas heartworm antigen was not detected in this study (95% CI; 0.0 - 1.4%). Out of these tested cats, 0.4% (95% CI; 0.1 - 2.1%) was found co-infected with both FeLV and FIV. The spatial distribution of retroviral infections in cats from 5 regions of Bangkok and Samut Prakarn province is shown in Table 5. The age of cats with seropositive for FeLV and FIV was ranged between 6 months and 8 years and between 6 months and 12 years old, respectively. There was no association between sex, ability to access outdoors and retroviral infections using Chi square test.

To test if retrovirus infections could possibly be one of the factors contributed to endoparasite infection in cats, 65.4% (176/269) of retrovirus-tested samples pertaining fecal examination results were tested for association between retroviral status and enteric parasite infections using Chi square test. The results revealed that no association between FIV, FeLV and any endoparasite infection.

3.4 Risk factors associated with hookworm infections

Table 5 Seroprevalence of FeLV, FIV and heartworm infections in cats in Bangkok and vicinities, 2014-2015 (n = 269)

Provinces	Regions	% of tested samples (n)	% seropositive (positive samples/tested samples)		
			FeLV	FIV	*Dirofilaria immitis*
Bangkok	**Northern**	34.6 (93)	8.6 (8/93)	7.5 (7/93)	0.0 (0/93)
	Eastern	31.6 (85)	9.4 (8/85)	5.9 (5/85)	0.0 (0/85)
	Southern	13.4 (36)	5.6 (2/36)	5.6 (2/36)	0.0 (0/36)
	Northern Thonburi	0.7 (2)	0.0 (0/2)	0.0 (0/2)	0.0 (0/2)
	Southern Thonburi	14.5 (39)	2.6 (1/39)	0.0 (0/39)	0.0 (0/39)
Samut Prakan	**Mueang Samut Prakan**	5.2 (14)	0.0 (0/14)	0.0 (0/14)	0.0 (0/14)
	Total	100 (269)	7.1 (19/269)	5.2 (14/269)	0.0 (0/269)

Risk factors associated with hookworm infections

From the univariate analysis, risk factors associated with *Ancylostoma* spp. infection are shown in Table 6. The multicollinearity was significantly found between ability to access outdoors, expose to other cats outside and expose to other dogs outside. So only ability to access outdoors was included in logistic regression. Significant factors including ability to access outdoors, history of vomiting/diarrhea, history of segment and/or adult worm presenting in feces and rodent hunting were tested using logistic regression. The adjusted odds ratios are shown in Table 7. Two factors, ability to access to outdoors (adjusted OR = 3.22, 95% CI; 1.42 – 7.87) and history of segment and/or adult worm presenting in feces (adjusted OR = 3.31, 95% CI; 1.34 – 8.21), were significant using multiple logistic regression.

Table 6 Univariate logistic regression analysis of risk factors associated with *Ancylostoma* spp. infection in cats living in Bangkok and vicinities, 2014-2015

Factor	Data	Positive for *Ancylostoma* spp. (n)	Negative for *Ancylostoma* spp. (n)	Odds ratio	95 % CI of odds ratio	*p*-value
Ability to access outdoors	Yes	46	97	3.06	1.48 – 6.32	0.002*
	No	11	71	ref		
Multi-cat household	Yes	33	105	0.79	0.43 – 1.48	0.46
	No	23	58	ref		
Living with dogs in the same household	Yes	18	52	1.01	0.53 – 1.94	0.97
	No	38	111	ref		
Expose to other cats outside	Yes	29	57	2.29	1.20 – 4.35	0.01*
	No	22	99	ref		
Expose to other dogs outside	Yes	26	40	3.02	1.56 – 5.81	0.001*
	No	25	116	ref		
Monthly routine deworming	Yes	7	14	1.48	0.57 – 3.87	0.42
	No	51	151	ref		
Monthly ectoparasite prevention	Yes	10	32	0.89	0.41 – 1.95	0.77
	No	47	134	ref		
Presence of flea	Yes	20	52	1.31	0.68 – 2.52	0.42
	No	30	102	ref		
History of diarrhea presenting	Yes	19	38	1.64	0.84 – 3.17	0.15*
	No	37	121	ref		
History of segment and/or adult worm presenting in feces	Yes	13	14	4.09	1.71 – 9.76	0.001*
	No	25	110	ref		
History of vomiting	Yes	29	83	1.03	0.56 – 1.91	0.91
	No	26	77	ref		
Rodent hunting	Yes	29	68	1.54	0.83 – 2.86	0.18*
	No	25	90	ref		
Reptile hunting	Yes	19	43	1.45	0.75 – 2.81	0.27
	No	35	115	ref		

Insect hunting	Yes	21	66	0.89	0.47 – 1.67	0.71
	No	33	92	ref		
Bird hunting	Yes	8	19	1.27	0.52 – 3.10	0.60
	No	46	139	ref		
Drinking water from natural sources	Yes	27	46	2.57	1.34 – 4.92	0.004*
	No	24	105	ref		

Table 7 Multivariable logistic regression analysis of risk factors associated with *Ancylostoma* spp. infection in cats living in Bangkok and vicinities, 2014-2015

Factors	Adjusted odds ratio	95 % CI of odds ratio
Ability to access outdoors	3.22	1.42 – 7.87
History of segment and/or adult worm presenting in feces	3.31	1.34 – 8.21

Descriptive statistics of with non-hookworm parasitic infections

There was an association between ability to access outdoors and any enteric parasitic infection status using Chi-square test ($p < 0.05$). Apart from association between hookworm and risk factors, the following is descriptive analysis of possible factors related to other parasites. Out of 509 tested fecal samples, 35 cats were positive for *Toxocara* spp. but around half of them, 18 cats, had data showing their age ranged between 5 months to 4.5 years. For lifestyle, 77.8% (14/18) of roundworm-infected cats could access to outdoors and 44.4% (8/18) of them exposed to cats outside. Half of roundworm-positive cats (9/18) lived in multi-cat household. The history of having a segment or adult of worm in feces was found in 33.3% (6/18) of them while 44.4% (8/18) and 27.8% (5/18) cats had a history of vomiting and diarrhea, respectively.

For *Cystoisospora* spp., out of 19 coccidia-positive cats, 36.8% (7/19) of them had complete info. and their ages were between 1 to 2 years of age. 71.4% (5/7) of them lived with other cats in the same household. 42.9% (3/7) of infected cats had contact with cats outside and the same percentage had a history of diarrhea within 6 months. 57.1% (4/7) of coccidia-infected cats had history of mouse hunting.

Out of 9 *P. fastosum*-positive cats with history recorded, their age was between 4 to 6 years. Around half of them, 55.6% (5/9), had access to outdoors and 22.2% (2/9) of liver fluke-infected cats had small reptile hunting behavior. Out of 2 flea tapeworm-positive cats, only one cat with history recorded and she was 6 months of age with lifestyle of strictly indoors and had fleas. From 15 rat tapeworm-positive cats, 4 cats had recorded history and they were 1 year old. Three quarters, 75% (3/4), had access to outdoors. Half of them (2/4) had rodent hunting behavior and had a history of having worm segment and/or adult worm in feces. From 8 *Spirometra*-positive cats, 2 cats with recorded history were 10 months of age and one of them had access to outdoors without frog and fish hunting behavior. Unfortunately, for *Opisthorchis*-like positive cat, we were unable to retrieve the history from his/her owner.

Discussions

This study reveals the latest prevalence data of cats living in Bangkok and vicinities. The strength of this study is that all samples were directly and freshly collected from cats thus making the results more relevant and reliable than those collected from environment where cats may roam (Table 8). Based on previous study, as stray dogs and cats shared the roaming areas in communities and Thai temples, we believe that small piles of puppy's or small-breed dog's feces could be mistaken as cat feces which found in the same living areas. In this study, none of free-living parasites in environments contaminated in our samples as fresh feces was directly collected from cat's anus making diagnosis affirmative as true cat endoparasites. Although this cat population may not serve as truly representative of all cats living in Bangkok metropolitan, convenience sampling can still reflect how wormy they were, about one third of tested cats, despite having someone to interact and take care of them. Our results reflect that owned or community cats can easily acquire infective stage of enteric parasites especially if they can access outdoors for roaming and hunting without routine deworming. Most importantly, these cats share the same environment with people hence serving as reservoir hosts of zoonotic parasites.

In line with almost all prevalence studies in Thailand and other Southeast Asian countries (Table 8), *Ancylostoma* spp. was the most predominant, 21.6% of tested cats, whereas previous prevalence was ranged between 3.0 and 78.0%. In Malaysia and Indonesia, *Ancylostoma* spp. was commonly found with prevalence 37.9 - 78.0% (Margono et al., 1979a; Ngui et al., 2014). From our study, hookworm in these cats were mostly subclinical infection serving as a reservoir to spread the infective stage into the environment wherever they roam and defecate resulting in considering as neglected zoonosis disease in Southeast Asia (Traub, 2013). In Thailand, *Ancylostoma caninum*, and *A. ceylanicu*m were reported using adult morphology and molecular identification (Setasuban et al., 1976; Pumidonming et al., 2017). Based on undistinguishable morphology of their eggs, the polymerase chain reaction technique can be used to identify at the species level (Palmer et al., 2007; Traub et al., 2008). This molecular identification is being conducted in our laboratory to identify these hookworms (manuscript in preparation).

In contrast to the prevalence of enteric parasites in cats from Europe that *Toxocara* spp. is the most found (Beugnet et al., 2014), prevalence of roundworm commonly found in kitten may be underestimated in this study since a small population of cats below 6 months old, 6.2% (14/227) of cats with complete questionnaire, were tested. Based on the morphology of roundworm egg, we did not specify species as *T. cati* since we aware that, apart from *T. cati*, *Toxocara malaysiensis* might be present in Thailand. *T. malaysiensis* was reported in Malaysia, Vietnam and China (Gibbons et al., 2001; Li et al., 2006; Anh et al., 2016; Le et al., 2016). So, further study to identify the species of these roundworms from our samples will be determined and to see if any and there will be any of clinical significance.

Since both hookworm and roundworm are soil-transmitted nematodes and potentially zoonotic (Bethony et al., 2006), for the cats raising outdoors, awareness of routine deworming as well as control and prevention should be emphasized to the cat owners (Dantas-Torres et al., 2020) to prevent human from cutaneous larva migrans, ocular and visceral larva migrans due to infection of these parasites especially in preschool children (Overgaauw and van Knapen, 2013). In Thailand, in

spite of the fact that selamectin spot-on and pyrantel embonate/praziquantel has been launched and commercially available since 2003 and 2008, respectively, the tendency of helminth infections in cats does not seem to be reduced (Table 8). In this population, only 9.3% (21/227) of cats had a history of monthly deworming but hookworm, roundworm and rat tapeworm were still present apart from coccidia and *Platynosomum* in some of these cats. So responsible pet ownership and client compliance should be highly encouraged to make sure that all cats are not only fed but also receive a wellness care including routine endoparasite and ectoparasite control.

Significant risk factors associated with hookworm infection in this study were ability of cats to access outdoors. Roaming cats can acquire infective stage (L3) of hookworm via direct cutaneous penetration of larva and consumption of larva-containing tissues of small vertebrate hosts or cockroach as well as consumption of L3-contaminated food from environment (Bowman et al., 2010). Another risk factors for *Ancylostoma* spp. infection was the history of segment and/or adult worm presenting in feces. Visible parts of helminths that can be recognized by owners are segments of cestodes and whole body of roundworm. *D. caninum* or *T. taeniaeformis* can be present motile around anus or in feces (Rodan and Sparkes, 2012). The latter and roundworm can also be expelled via vomitus. Consequently, cat lifestyle and related history of helminth presence in feces should be included for history taking from clients to support the possibility of hookworm infection.

For retroviral infections, current study revealed lower prevalence of both retroviral infections compared to previous reports (Nedumpun et al., 2015; Sukhumavasi et al., 2012). Since the majority of samples obtained from previous studies were collected from hospitals, veterinary clinics and a diagnostic center, cats with clinical diseases may be included hence resulting in higher prevalence. In contrast, cats recruited to our study were mostly brought for neutering service, so they were most likely more clinically healthy. Also, FeLV antigen testing does not detect the regressive infection so infected cats may still be underestimated (Sykes and Hartmann, 2014). Owned or community cats with access to outdoors are riskier to be infected due to higher opportunity to interact with other infected cats (Sykes

and Hartmann, 2014) but our study did not find the significant difference of FeLV/FIV infection between outdoor and indoor cats. For the age of retrovirus-infected cats, FeLV- and FIV-infected cats were between 6 months to 8 years and 6 months to 12 years, respectively. This suggested that retrovirus-infected cats can live long if they receive good wellness care to prevent from opportunistic infection from their owners. About sex, we found FIV-infected cat were 57.1% (8/14) male and 42.9% (6/14) female in which there was no difference for FIV infection. So, this result was not in line with previous study that male was a risk factor for FIV infection (Sukhumavasi et al., 2012). For the finding of no association between FIV, FeLV and any endoparasite infection, if there are more numbers of FeLV- and FIV-seropositive cats, we expect to see significant difference.

As comparative prevalence of endoparasites in cats living in Bangkok and Nonthaburi is shown in Fig. 1, hookworm infection was the most common in both provinces. However, *Taenia taeniaeformis* was found as the 2nd rank in Nonthaburi, 5.2%, whereas this tapeworm was found as the 5th rank in Bangkok, 2.1%. This may be due to more favorable habitats for rodent population growth in Nonthaburi associated with orchards and rice fields to serve as development of strobilocercus in intermediate rodent host. In Fig. 2A and 2B, hookworm was the most common in all regions of Bangkok and almost all districts of Nonthaburi except for Mueang Nonthaburi that roundworm was outnumbered. If high proportion of kittens happened to be collected from this district, it may be possible to detect higher number of roundworm-infected kittens. However, age record of these roundworm-infected cats was unavailable so being unable to explain this difference.

Despite the ability to access outdoors considering as one of the risk factors for hookworm infection, interestingly, even in the strictly indoor cats, parasites were still detected. Out of strictly-indoor cats tested, *Ancylostoma* spp. was the most found 13.4% (11/82), followed by *Toxocara* spp. 4.9% (4/82) and *Platynosomum fastosum* 3.7% (3/82). *Dipylidium caninum*, *Taenia taeniaeformis*, *Spirometra* spp. and *Cystoisospora* spp. were independently found 1.2% (1/82) from different cats. This may be due to these cats being infected prior to being adopted from stray cats and later become exclusively indoors lifestyle but has not yet been dewormed. Our

questionnaire should have been better designed to distinguish such cases in order to explain parasitic infection in indoor cats. Regarding the history of anti-parasitic product usage, 18.2% (2/11) of hookworm-infected indoor cat and 16.7% (3/18) roundworm-infected indoor cats had history of receiving endoparasite preventatives. For tapeworm-positive indoor cats, 25.0% (1/4) cats used ectoparasite control product. Despite using anthelminthic products but still had positive results, one of possible explanations could be due to hookworm larva hypobiosis and become re-activated post deworming then migrate from tissues and become patent infection (Schneider et al., 2011).

For other possibilities that indoor cats can be infected with endoparasites, paratenic or intermediate hosts of these parasites may be sneaked into the house and got hunted. According to previous reports, *Ancylostoma caninum* and *T. cati* can be transmitted via cockroaches and rodents, respectively (Bowman et al., 2010; Holland, 2017). For flea tapeworm, undoubtedly, cysticercoid-containing fleas can colonize and live indoors. Once they become mature, adult fleas can be taken up by these indoor cats (Sapp and Bradbury, 2020). So monthly use of ecto- and endoparasite preventatives should be applied continuously until the fecal test becomes not found and evaluated periodically (Dantas-Torres et al., 2020).

For protozoan infection, as most of these cats looked clinically healthy and not diarrheic, we did not detect trichomonad or *Giardia* trophozoite and cyst by conventional fecal examination method. However, copro-antigenic testing of *Giardia* cyst wall protein yielded prevalence at 3.3% (9/233) of tested cats. This may be due to high sensitivity of the commercial test to potentially identify asymptomatic or reservoir cats. Also, since *Giardia* cysts are intermittently shed (Vasilopulos et al., 2006), collection feces at least three times within a week to be examined using $ZnSO_4$ centrifugal flotation before ruling this infection out is recommended. To determine zoonotic assemblages, *Giardia* antigen-positive samples need to be subjected to PCR (Thompson and Ash, 2016; Caccio et al., 2018).

Combined multiple diagnostic techniques for endoparasite detection can improve sensitivity of detection. Wet fecal smear is useful for screening test whereas flotation and sedimentation are suitable concentration technique for non-

operculated egg/oocyst/cyst and operculated egg in addition, respectively (Dantas-Torres et al., 2020). Sedimentation was selected to be prioritized technique in our study as this method can cover both non-operculated nematode eggs, oocyst as well as operculated liver fluke egg that may be neglected but important in cats living in tropical regions (Shell et al., 2015). When we compared the differences between each coprological technique for testing the same sample, $ZnSO_4$ centrifugal flotation and centrifugal sedimentation were suitable for hookworm egg detection whereas only $ZnSO_4$ centrifugal flotation was suitable for *Cystoisospora* oocyst detection. The latter was in line with previous study (Dryden et al., 2005). For platyhelminths, interestingly, operculated eggs of *Spirometra* spp. and *Platynosomum fastosum* were also recovered by using flotation technique. For small liver fluke egg detection, centrifugal sedimentation was recommended (Basu and Charles, 2014). However, from our study, detection rate between PBS-ethyl acetate centrifugal sedimentation and $ZnSO_4$ centrifugal flotation was not significantly different (p = 0.06). This may be due to the too few positive sample size numbers. We would expect to see the significant difference if positive samples are greater than this.

Apart from *Platynosomum fastosum*, this identified another trematode egg as *Opisthorchis*-like egg. Since the morphology of minute intestinal fluke egg and *Opisthorchis viverrini* egg are similar and the latter is more common in the northeastern part of Thailand (Aunpromma et al., 2012), further characterization by molecular technique (Enes et al., 2010; Buathong et al., 2017) or deworming to expel adult for morphological identification can be performed for definitive diagnosis. Various species of minute intestinal flukes belonging to family Heterophyidae were reported from stray cats living in the riverside area in Korea such as *Metagonimus* spp., *Pygidiopsis summa*, *Heterophyes nocens*, *Stellantchasmus falcatus*, *Heterophyopsis continua*, *Acanthotrema felis*, *Centrocestus armatus*, *Procerovum varium, Cryptocotyle concava*, and *Stictodora lari* (Shin et al., 2015). To the authors' knowledge, there were 2 reports on minute intestinal fluke of cats in Thailand in which the genus has not been identified (Muksombat et al., 2008; Enes et al., 2010).

For pseudophyllidean tapeworm, *Diphyllobothrium* spp. and *Spirometra* spp. can be found in cats. Based on morphology of irregular egg shell, asymmetrical

appearance and pointed at one end (Bowman et al., 2008), *Spirometra* spp. was identified in our study. In other Asian countries, *Spirometra erinaceieuropaei* and *Diphylobothrium nihonkaiense* were reported in Japan (Yamamoto et al., 2009) while *Spirometra decipiens* was reported in Korea (Jeon et al., 2018). For cyclophyllidean tapeworm, *Dipylidium caninum* proglottid needs to be distinguished from *Joyeuxiella* spp. that had a report in a cat in Thailand (Chungpivat et al., 2004). For egg packet, they were confirmed in this study based on more than 5 eggs/egg capsule (Bowman et al., 2008).

Regarding the limitation of this study, since majority of fecal samples were directly collected from cats during post-operation recovery thus making the mass of feces somewhat limited due to fasting overnight before anesthesia. Out of 509 samples collected, fecal samples were obtained per rectum 98.4% (501/509) and by colon flushing 1.6% (8/509). By colon flushing, only centrifugal sedimentation technique can be performed. Despite limited fecal mass by colon flushing, flushed excreta can yield 12.5% (1/8) of flushed sample to be positive for *Ancylostoma* spp. However, even with this limitation, endoparasites were detected from one third of these tested cats reflecting that endoparasitic infections are most likely still be underestimated.

Conclusion

This work revealed that infections with helminths and coccidia are common in cats living in Bangkok and vicinities. Out of 509 samples, *Ancylostoma* spp. was found as the first rank, 21.6%, followed by 6.9% *Toxocara* spp., 3.7% *Platynosomum fastosum*, 3.5% *Cystoisospora* spp., 2.9% *Taenia taeniaeformis*, 1.6% *Spirometra* spp., 0.4% *Dipylidium caninum* and 0.2% *Opisthorchis*-like trematode egg. For retroviruses, FeLV and FIV were positive 7.1% (19/269) and FIV 5.2% (14/269), respectively, without association with endoparasitic infection. Based on *Giardia* copro-antigen detection test, 3.9% (9/233) of tested cats were positive. From multivariable logistic regression, ability to access outdoors and having segment or adult worm in feces were significantly associated with *Ancylostoma* spp. infection. Since hookworm and roundworm are zoonotic, so emphasis on deworming should

be encouraged especially in endemic area and routine fecal examination with appropriate technique should be performed before ruling out parasitic infection.

Acknowledgments

This research was partially funded to WS by The Chulalongkorn University Research Unit for Feline Infectious Disease and Health for Excellence, The Chulalongkorn University Research Unit for Microbial Food Safety and Antimicrobial Resistance, The Chulalongkorn University Research Unit (GRU 6203331007-1) and to WJ by The 100th Anniversary Chulalongkorn University for Doctoral Scholarship and The 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). Authors would like to gratefully thank Prof. Dr. Chollada Buranakarl and the Department of Livestock Development for the opportunity to join the public veterinary services. Moreover, authors would like to thank undergraduate student volunteers (Punyahathaikul T., Sukkasemsuwan S., Rangsiyanan R., Phanpongpipat N., Thongmeesee K.), and graduate students in parasitology unit, Faculty of Veterinary Science, Chulalongkorn University for cooperating in sample collection and processing.

Chapter 3

Molecular identification and characterization of feline hookworm and *Giardia* spp. in Bangkok and vicinities, Thailand

Wanarit Jitsamai[a], Laura del Río[b], Tawin Inpankaew[c], Woraporn Sukhumavasi[a]*

[a]Parasitology Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University Bangkok 10330, Thailand

[b]Department of Animal Health, Faculty of Veterinary, Mare Nostrum International Excellence Campus, University of Murcia, Spain

[c]Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand.

*Corresponding

E-mail address: vetkwan@hotmail.com

Keywords:

Cats, Hookworm, Giardia, Molecular Identification, Bangkok, Thailand

Abstract

Hookworm is one of the most common parasites found in cats in Thailand. However, there was no molecular identification at the species level to relate hookworm with zoonotic infection. Also, *Giardia* assemblage never been reported in cat in this region. To address the role of cats in parasitic zoonotic transmission, species of hookworm and *Giardia* assemblage were determined. From a total of 207 hookworm positive sediment samples collected in 2014-2016 and subjected to PCR amplifying *ITS1*, *5.8S* and partial *ITS2* regions, 59.9% (124/207) was positive and further submitted to sequencing. Sixty-four sequences were analyzed using phylogenetic tree analysis. Out of 64 sequences obtained, 98.4% (63/64) were identified as *A. ceylanicum* and 1.6% (1/64) was found as *A. tubaeforme*. *A. ceylanicum*-positive samples were selected to amplify *COX1* gene for characterization at a clade level. All them were grouped into a clade of *A. ceylanicum* from cats that is different from human clade suggesting that *A. ceylanicum* from cats collected in this study was most likely have a low potential in zoonotic transmission. For *Giardia* molecular identification, a total of 304 DNA samples was grouped in a pool of 4 samples and were tested with nested PCR targeting *SSU rRNA* gene of *Giardia*. Only 1 sample was positive. Based on phylogenetic tree analysis, *Giardia* assemblage D was confirmed in which this assemblage is commonly found in dog supporting the possibility for *Giardia* circulate between cats and dogs. Since this study reports *A. tubaeforme* for the first time in Thailand, hookworm infection should be recognized in clinical practice due to their ability to consume much blood volume than other hookworm species. Also, *A. ceylanicum* the most predominant hookworm in cats in Thailand that can develop into adult in human. So, monthly prevention and control of hookworm is encouraged in endemic country.

Introduction

Hookworm is the most predominant enteric parasites found in cats in Thailand (Jittapalapong et al., 2007a; Rojekittikhun et al., 2013; Rojekittikhun et al., 2014). Cats are considered a definitive host for various species of hookworm including *Ancylostoma tubaeforme, A. braziliense, A. ceylanicum* and *Uncinaria stenocephala* (Bowman et al., 2010). Two species of hookworms, *A. caninum* and *A. ceylanicum* were reported in cats in Thailand, whereas 92-96% of hookworm-infected cats in Thailand were infected by *A. ceylanicum* (Setasuban et al., 1976; Pumidonming et al., 2017), recognized as a re-emerging nematode (Traub, 2013). The recent study also revealed *A. ceylanicum* infection in humans from Ubon Ratchathani province, Thailand (Niamnuy et al., 2016).

Hookworm can cause several clinical manifestations such as acute and chronic blood loss mainly caused by *A. caninum, A. tubaeforme* and *A. ceylanicum*. The human can acquire these parasites by penetration of infective larvae through the skin and leave a tract-like lesion called cutaneous larva migrans (CLM) or creeping eruption, in which it is caused by inflammatory responses making patients intensely pruritic. Larvae can be found trapped in the epidermis of the tract-like lesions and die within 5-6 weeks (Tremblay et al., 2000). CLM in human can be found in tourists associated with the previous history of traveling to Thailand (Caumes et al., 2002; Nakamura-Uchiyama et al., 2002; Miesen, 2003; Malvy et al., 2006; Morsy et al., 2007; van Nispen tot Pannerden et al., 2007; Feldmeier and Schuster, 2012; Veraldi et al., 2013; Creamer, 2014). Moreover, *A. ceylanicum* can penetrate the skin and migrate to the small intestine to develop into adults in humans (Prociv, 1997).

For zoonotic protozoa, while the majority of Giardia assemblage in stray dogs living in Thai temples was documented as assemblage A (Inpankaew et al., 2007). Giardia assemblage A and B have a wide-range host including humans, dogs and cats so they are responsible for diarrheic illness in humans. The assemblage of this protozoan in cats has not yet been explored. PCR can be used for species identification of hookworms as well as Giardia assemblages. So, the prevalence of zoonotic hookworms, *A. ceylanicum* and *Giardia* assemblage A and B should be

determined to evaluate the role of cats as reservoirs for potentially zoonotic enteric parasites.

Materials and Methods

Sample collection, parasitological procedures and DNA extraction

Cat fecal samples were collected by rectum and/or colon flushing during post-neutering recovery from in Bangkok and vicinities, Thailand, between 2014 and 2016. All samples were proceeded with conventional microscopic examinations including fresh fecal smear, PBS-ethyl acetate centrifugal sedimentation and $ZnSO_4$ centrifugal flotation. Sediments were kept at -20°C until DNA extraction was performed. Approximately 200 µl of sediments were used for DNA extraction using QIAamp DNA Stool Mini Kit (Qiagen, USA) according to manufacturer's protocol with three repeated cycles of freezing (one minute in liquid nitrogen, -196°C) and thawing (five minutes boiling in a water bath).

PCR targeting internal transcribed spacer *ITS1*, *5.8S* and *ITS2* regions and cytochrome c oxidase subunit 1 (*COX1*) of hookworm

The species of hookworms were identified using conventional PCR targeting 545 bp of partial *ITS1, 5.8S* and partial *ITS2* regions of *A. caninum, A. tubaeforme, A. ceylanicum* and *Uncinaria stenocephala* followed by sequencing. Forward primer RTGHFI and reverse primer RTABCR1 (Palmer et al., 2007) were used. A total of 25 µl reaction consisted of 4.0 µl of DNA sample, 12.5 µl 2x PCR master mix solution (i-Taq™, Intron Biotechnology, Korea), and 0.5 µM of each primer. Thermocycler was set for 35 cycles of 94°C for 20s, 64°C for 10s, and 72°C for 40s with 94°C for 2 min of initial denaturation and 72°C for 5 min of final extension.

Negative samples or positive samples that sequences were not obtained were proceeded with PCR targeting *ITS1*, *5.8S* and *ITS2* regions and subjected to amplification of partial *ITS2* with the expected product of 400 bp using forward primer NC1and reverse primer NC2 (Gasser et al., 1993). A total of 25 µl reaction of PCR component was described above. Thermocycler was set for 35 cycles of 94°C for

45s, 50°C for 45s, and 72°C for 45s with 94°C for 7 min of initial denaturation and 72°C for 10 min of final extension. All primers used were listed in Table 1.

A. ceylanicum-positive sample was characterized in clade level by PCR targeting mitochondrial gene, *COX1*, with the expected product of 377 bp using forward primer AceyCOX1F and reverse primer AceyCOX1R (Inpankaew et al., 2014a). A total of 25 µl reaction of PCR component was described as above. Thermocycler was set for 50 cycles of 94°C for 30s, 58°C for 30s, and 72°C for 30s with 94°C for 5 min of initial denaturation and 72°C for 7 min of final extension.

All PCR products were analyzed on 1.5% agarose gel electrophoresis with SYBR® Safe DNA Gel Stain (Invitrogen, CA, USA) and visualized under transilluminator followed by sequencing. Positive samples were selected and submitted to commercial sequencing (Macrogen, Inc., Korea).

Nested PCR targeting small subunit ribosomal RNA gene (*SSU rRNA*) of *Giardia* spp.

Giardia DNAs were detected and characterized using nested PCR by targeting *SSU rRNA* gene (Inpankaew et al., 2014b). All samples were subjected to DNA extraction using QIAamp DNA Stool Mini Kit (Qiagen, USA). For the primary PCR, the expected PCR products of 292 bp were amplified using forward primer RH11 and reverse primer RH4. A total of 25 µl reaction of PCR components was described as above with 1.0 µl of DNA template and 0.4 µM of each primer. Thermocycler was set for 35 cycles of 94°C for 20s, 55°C for 10s, and 72°C for 30s with 94°C for 2 min of initial denaturation and 72°C for 3 min of final extension. Products from primary PCR were diluted 1:10 with sterile distilled water and used as a template in secondary PCR. For the secondary PCR, the expected PCR products of 174 bp were amplified by using forward primer GiaF and reverse primer GiaR. A total of 25 µl reaction of PCR components was the same as primary PCR. Thermocycler was set for 35 cycles of 94°C for 20s, 58°C for 10s, and 72°C for 30s with 94°C for 2 min of initial denaturation and 72°C for 3 min of final extension. The final PCR products were proceeded as described above. All primers used were listed in Table 8.

PCR targeting *16S rRNA* gene of enteric bacteria as an internal control

All samples were proceeded with PCR targeting *16s rRNA* gene of enteric bacteria by using universal primer, forward primer 515F and reverse primer 1391R (Gookin et al., 2007) to verify the quality of DNA samples. A total of 25 µl reaction of PCR components was described as above. Thermocycler was set for 35 cycles of 94°C for 30s, 64°C for 30s, and 72°C for 60s with 94°C for 2 min of initial denaturation and 72°C for 7 min of final extension. All primers used were listed in Table 8

.

Table 8 Primer sets used for detection of cat hookworm and *Giardia* in this study.

Name	Sequences	Expected product size (bp)	References
RTGHFI	5′-CGTGCTAGTCTTCAGGACTTTG-3′	545	(Palmer et al., 2007)
RTABCR1	5′-CGGGAATTGCTATAAGCAAGTGC-3′		
NC1	5′– ACGTCTGGTTCAGGGTTGTT–3′	400	(Gasser et al., 1993)
NC2	5′– TTAGTTTCTTTTCCTCCGCT – 3′		
AceyCOX1F	5′-GCTTTTGGTATTGTA-AGACAG-3′	377	(Inpankaew et al., 2014a)
AceyCOX1R	5′- CTAACAACATAATAAG-TATCATG-3		
RH11	5′-CATCCGGTCGATCCTGCC-3′	292	(Inpankaew et al., 2014b)
RH4	5′-AGTCGAACCCTGATTCTCCGCCAGG-3′		
GiaF	5′-GACGCTCTCCCCAAGGAC-3′	174	(Inpankaew et al., 2014b)
GiaR	5′-CTGCGTCACGCTGCTCG-3′		
515F	5′–GTGCCAGCAGCCGCGGTAA–3′	1000	(Gookin et al., 2007)
1391R	5′–GACGGGCGGTGAGTGC A–3′		

Statistical analysis

Cohen's kappa coefficient was used to determine the agreement between the results from PCR targeting bacterial *16S rRNA* and both PCR targeting hookworm DNA.

Results

Molecular characterization of hookworm using PCR targeting *ITS1*, *5.8S* and partial *ITS2* regions

Based on a total of 835 cat fecal samples collected in 2014-2016 from Bangkok and vicinities, Thailand, 34.9% (291/835) of them were positive for hookworm using at least one microscopic conventional fecal examination technique. Only 207 hookworm-positive samples with remaining sediments were subjected to PCR amplification for partial *ITS1*, *5.8S* and *ITS2* regions of *Ancylostoma caninum*, *A. tubaeforme, A. ceylanicum* and *Uncinaria stenocephala*. There were 30.9% (64/207) of them positive for conventional PCR and a subset of them, 48.4% (31/64), was submitted to sequencing. The phylogenetic tree from the sequences of partial *ITS1*, *5.8s* and *ITS2* was constructed using Maximum likelihood methods with Kimura 2-parameter for substitution model. The result revealed that all samples were grouped in the cluster of *Ancylostoma ceylanicum* (GenBank accession no. KC755027, JQ812694 and KP844730) (Figure 3).

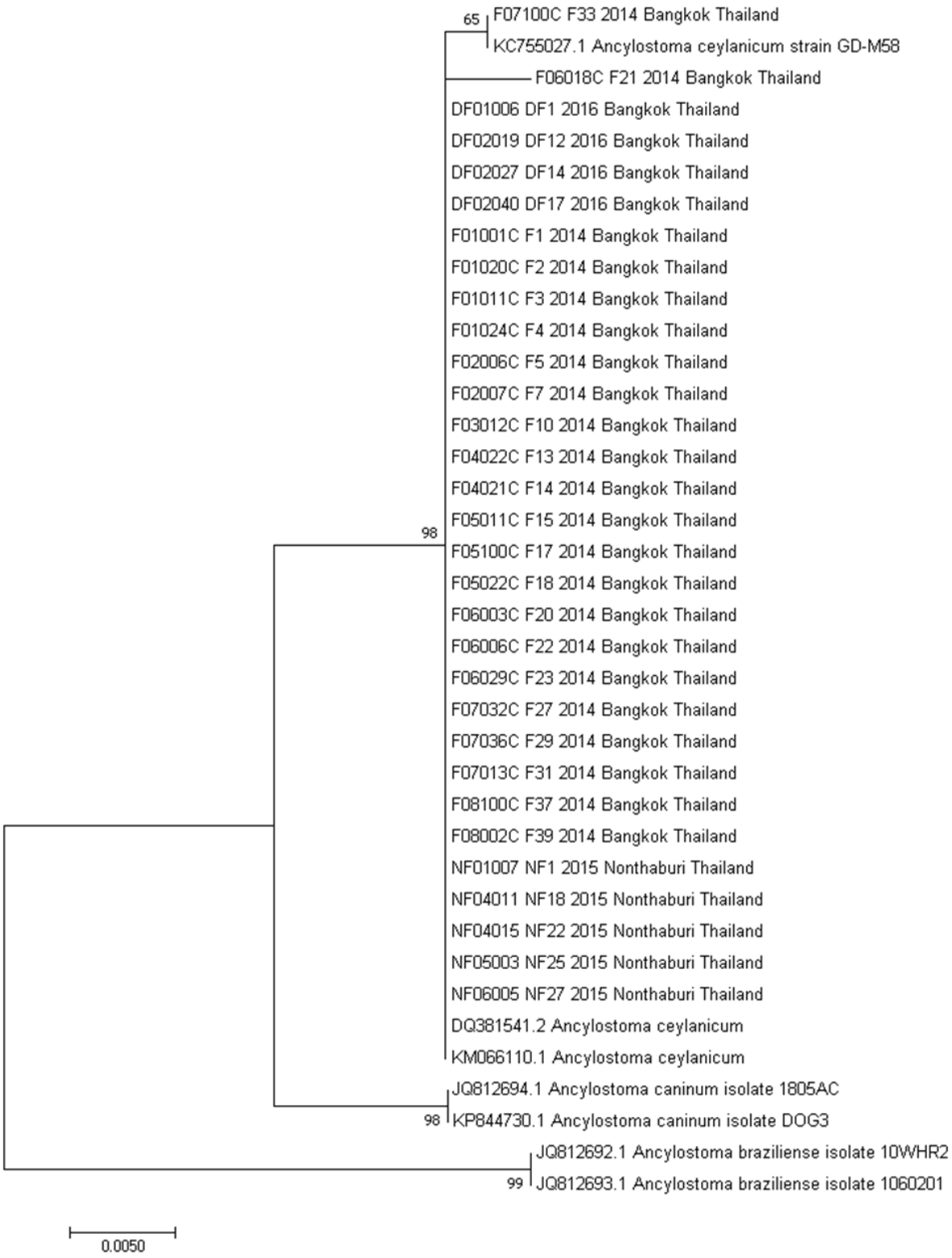


Figure 3 Phylogenetic tree analysis of partial *ITS1, 5.8S* and *ITS2* gene of *Ancylostoma* spp.

A total of 143 negative samples and 33 positive samples that sequences were not obtained were subjected to PCR targeting partial ITS2 regions. Out of these samples, 43.8% (77/176) were positives and 33 out of 77 were submitted to sequencing. The phylogenetic tree from the sequences of partial ITS2 was constructed using Maximum likelihood methods with Kimura 2-parameter for substitution model. The phylogram revealed that 97% (32/33) were grouped with *A. ceylanicum* (GenBank accession no. KM066110) and one sample was grouped with *A. tubaeforme* (GenBank accession no. JQ812691) (Figure 4). Combining 2 PCR procedures, 59.9% (124/207) were positive for hookworm and characterized, 64 out of 124 samples reveals *A. ceylanicum* 33.4% (63/207) and *A. tubaeforme* 0.5% (1/207).

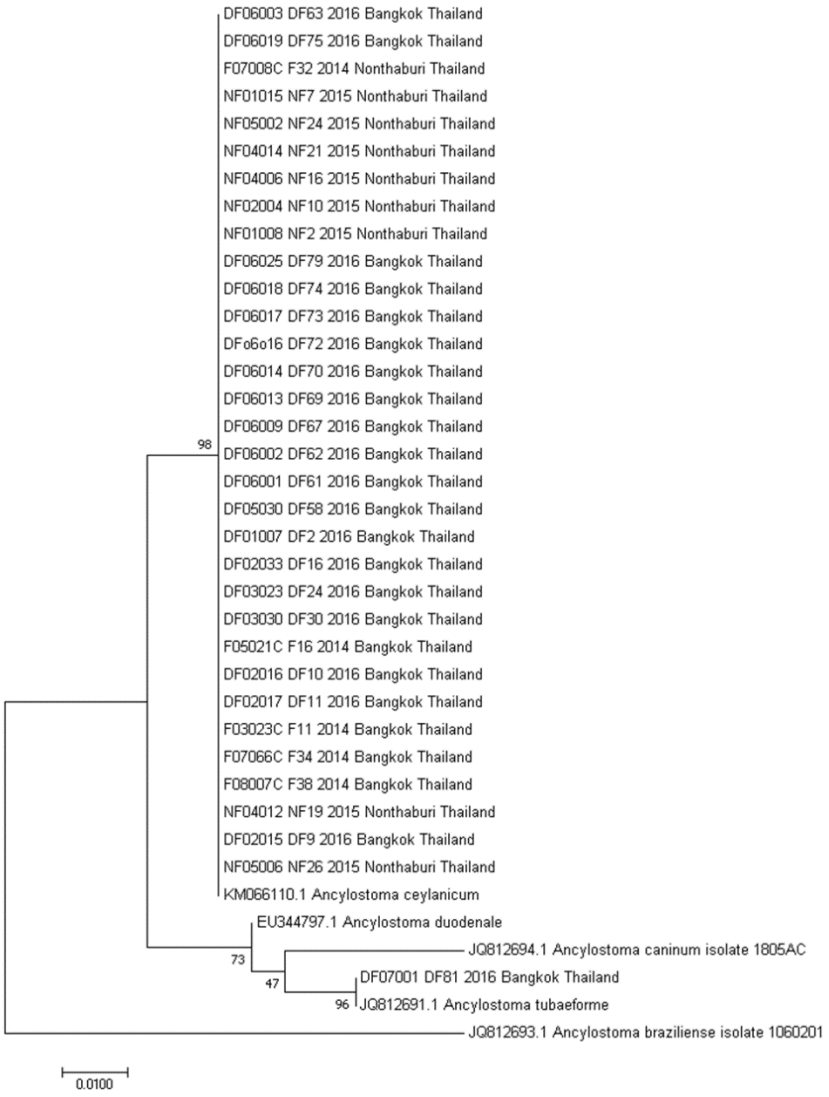


Figure 4 Phylogenetic tree analysis of partial *ITS2* gene of *Ancylostoma* spp.

A total of 22 *A. ceylanicum*-positive samples were selected to proceed with PCR amplifying *COX1* gene. There are 81.8% (18/22) positive and half of them (9/18) were selected for sequencing. The phylogenetic tree from sequences of *COX1* gene was constructed using Neighbor-joining methods with Kimura 2-parameter for substitution model. The phylogram revealed that *A. ceylanicum* were grouped into 2 clusters including human cluster (GenBank accession no. KC247745 and KF896605) and (GenBank accession no. KC247730, KF896595, KC896596, KC247737 and KF896601) and separated from *A. caninum* (GenBank accession no. NC012309) and *A. duodenale* (GenBank accession no. NC003415). A total of 9 sequences were grouped in mixture of human and dog clusters (Figure 5).

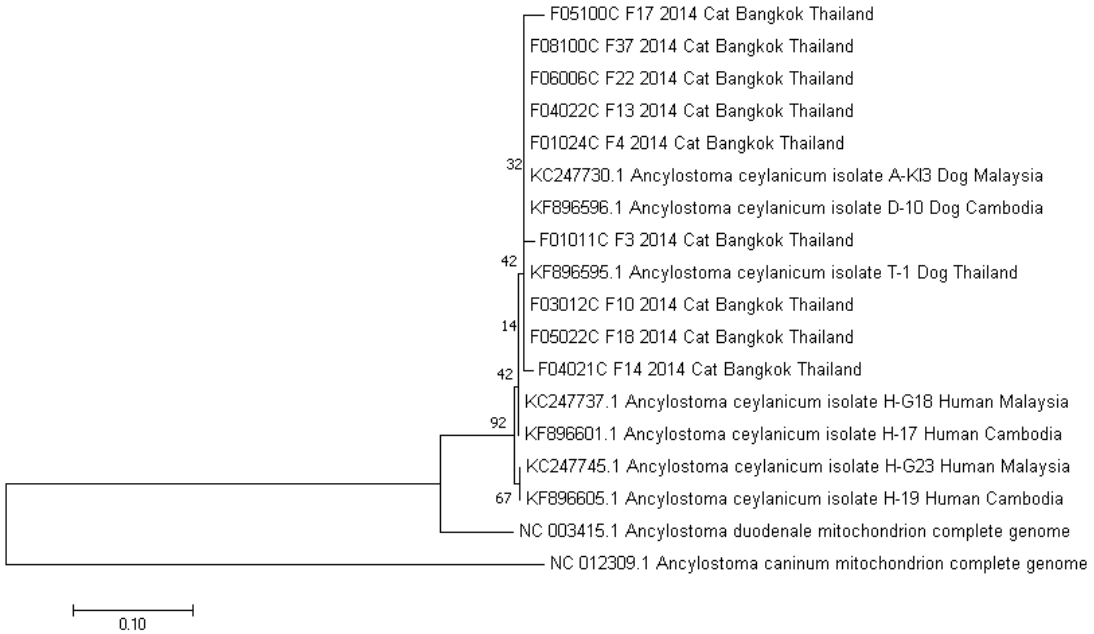


Figure 5 **Phylogenetic tree analysis of *COX1* gene of *Ancylostoma ceylanicum***

Molecular detection of *Giardia* spp. using nested PCR targeting *SSU rRNA*

A total of 304 DNA samples were grouped in a pool of 4 samples and were tested with nested PCR. Only one pool was positive and individually tested. One sample, 0.3% (1/304) was positive and submitted to sequencing. Phylogenetic tree was conducted using references including assemblage A (GenBank: AF199446), B (GenBank: AF199447), C (GenBank: AF199449), D (Genbank: AF199443), E (Genbank:

AF199448) and F (Genbank: AF199444) (Minetti et al., 2014). The result showed that a positive sample was assemblage D.

PCR targeting 16s rDNA gene of enteric bacteria as an internal control.

To ensure the quality of DNA extracted from all samples, they were subjected to PCR to amplify *16S rRNA* gene from enteric bacteria. There were 71.8% (148/206) of tested samples positive. Statistical analysis using Cohen's kappa coefficient revealed that the agreement between PCR targeting bacterial *16S rRNA* and PCR targeting hookworm *ITS1, 5.8S* and *ITS2* was fair with 53.4% of agreement (Cohen's k = 0.2). Also, the agreement between PCR targeting bacterial *16S rRNA* and PCR targeting hookworm *ITS2* was fair with 66.9% of agreement (Cohen's k = 0.4)

Discussions

By molecular identification and characterization, almost all hookworm collected from cats in this study from Bangkok and vicinities were identified as *Ancylostoma ceylanicum* which was in line with a survey conducted in the lower northern part of Thailand by molecular identification (Pumidonming et al., 2017) and by morphological identification (Setasuban et al., 1976). *A. caninum* and *A. braziliense* were not molecularly detected in this study despite including primer sets to detect both of them. Hookworm is worldwide distributed but not imperfect due to climate range overlap. *A. braziliense* is found along south Atlantic coastal regions of North America, the Caribbean Sea and South America. *A. caninum* and *A. tubaeforme* are found in warmer ranges (Bowman et al., 2010) while *A. ceylanicum* is found in Asia and Australia (Traub, 2013). *A. ceylanicum* is commonly found in cats especially in Asia including China, Laos, Malaysia, Taiwan and Thailand (Table 2). *A. ceylanicum* is a predominant hookworm in cats in Southeast Asia while *A. caninum* is more common than *A. ceylanicum* in China. Similarly, *A. ceylaninum* is predominant in dogs in Asia as well. Since PCR has been developed to identify the species of hookworm (Traub et al., 2004; Palmer et al., 2007), the hidden species of hookworm in humans were uncovered. Apart from *N. americanus* and *A. duodenale*, several zoonotic hookworms were recently documented. *A. ceylanicum* is considered as the

second common hookworm infection in humans in Asia (Thompson, 2015) and also the most neglected among human hookworms (Conlan et al., 2011). Although *A. ceylanicum* is not major causative agent of cutaneous larva migrans (Bowman et al., 2010), it is capable of developing into adult in human causing abdominal discomfort (Traub, 2013). There were reports found that clinical manifestations after chronic intestinal infection with *A. ceylanicum* included diarrhea, iron-deficiency anemia and protein-losing malnutrition (Carroll and Grove, 1986; Tu et al., 2008).

Table 9 Comparison of distribution of different hookworm species in cats in Asia by molecular and morphological identification

Location	Methods	n	A. ceylanicum (%)	A. caninum (%)	A. tubaeforme (%)	A. braziliense (%)	References
China	PCR	97[b]	40.2	95.1	0.0	0.0	(Liu et al., 2013)
China	PCR	49[b]	40.8	79.6	0.0	0.0	(Hu et al., 2016)
China	PCR	47[b]	51.1	0.0	48.9	0.0	(Fu et al., 2019a)
Taiwan	Necropsy	10[a]	30.0	0.0	10.0	0.0	(Yoshida et al., 1968)
Iran	Necropsy	50[a]	0.0	4.0	18.0	0.0	(Changizi et al., 2007)
Iran	Necropsy	100[a]	0.0	0.0	64.0	0.0	(Sharif et al., 2010)
Indonesia	n/a	50	24.0	0.0	2.0	72.0	(Margono et al., 1979b)
Laos	Necropsy	55[a]	69.0	0.0	2.0	0.0	(Scholz et al., 2003)
Malaysia	Necropsy	2[a]	Found [c]	0.0	0.0	Found [c]	(Yoshida et al., 1973)
Malaysia	PCR	7[b]	85.7	0.0	0.0	14.3	(Ngui et al., 2012)
Malaysia	Necropsy	543[a]	31.5	0.0	0.0	30.8	(Mohd Zain et al., 2013)
Malaysia	PCR	152[a]	29.6	6.6	0.0	0.0	(Tun et al., 2015)
Thailand	Necropsy	n/a	92.0	23.0	0.0	0.0	(Setasuban et al., 1976)
Thailand	PCR	25[b]	96.0	4.0	0.0	0.0	(Pumidonming et al., 2017)
Thailand	PCR	207[b]	30.4	0.0	0.5	0.0	This study

n/a not accessible.

[a]Number of overall samples

[b]Number of hookworm-positive samples

[c]The numeric data was not clarified by authors.

Since *A. braziliense* was not detected in this study and rarely found in Southeast Asia (Yoshida et al., 1973; Margono et al., 1979b). The incidences of CLM in this region were possibly caused by *A. ceylanicum* and *A. caninum* otherwise the geographic distribution of *A. braziliense* in Southeast Asia should be reassessed (Conlan et al., 2011).

High prevalence of *A. ceylanicum* in dogs and cats in Fujian province, China is recognized as a risk factor for human infection (Chen et al., 2012). Several studies suggested that dogs were responsible for reservoirs of the zoonotic *A. ceylanicum* cycle (Conlan et al., 2012; Inpankaew et al., 2014a; Thompson, 2015) but the role of cats has not been addressed yet. Characterization of *COX1* gene of hookworm from cats in this study revealed that cat hookworm in Bangkok and vicinities, Thailand, seemed to be distinguished from human hookworm. To confirm this hypothesis, sequences of *COX1* gene from cat hookworm should be analyzed along with sequences from human hookworm collected in the same area. In order to control human hookworm (Conlan et al., 2012), the role of animal reservoir especially pets having a close contact with their owners should be addressed in parallel with mass drug administration programs implementing on both humans and animals.

This study is the first report of *A. tubaeforme* in Thailand. *A. tubaeforme* is widely distributed including Australia, Brazil, Italy, Qatar, Spain, the United States of America and China (Fu et al., 2019b). Clinical signs of *A. tubaeforme* infection depends on the dose of infection. High dose of infection results in rapidly decreased pack cell volume and hemoglobin as well as weakness, lack of appetite, and tousled hair especially in 3 weeks after infection (Onwuliri et al., 1981; Vatta et al., 2019). *A. tubaeforme* is more pathogenic than *Uncinaria stenocephala* and *A. braziliense* as it consumes more blood than the others. This study suggests that the presence of hookworm eggs in pet or community cats should not be neglected. Apart from molecular identification, morphometric measurement can also be conducted to distinguish the egg of hookworm species (Lucio-Forster et al., 2012).

Giardia spp. is characterized using DNA-based techniques into assemblage A to H (Caccio et al., 2018). Cats can be infected with *Giardia* assemblage A, B and F in which these assemblages was proposed as *G. duodenalis*, *G. enterica* and *G. cati*,

respectively (Thompson and Ash, 2016). In Bangkok, previous study revealed that the majority of *Giardia* spp. from stray dogs in Thai temples was assemblage A (Inpankaew et al., 2007). Since then, the assemblage of cats in Thailand has not been determined. Interestingly, we found that only one positive sample was *Giardia* assemblage D. Similarly, assemblage D was found in cats in Chiang Mai, Thailand (Tangtrongsup et al., 2020). Apart from Thailand, *Giardia* assemblage D was reported in cats in Japan and Australia (Palmer et al., 2008; Ito et al., 2017). The possibility of this finding may be that cats could also be infected with *Giardia* assemblage D which commonly found in domestic and wild canids or simply taken up and expelled as spurious parasite. While cat-specific assemblage F and dog-specific assemblage C and D were occasionally reported in cattle and pig (Caccio et al., 2018), we hypothesized that cats potentially acquired assemblage D from dogs as well. Regarding parasite circulating between pet animals, another study revealed that dog hookworm in Bangkok is *A. ceylanicum* (Sukhumavasi et al., manuscript on preparation) which is commonly found in cats. Moreover, risk factors analysis demonstrated that dogs having a close contact with cats had higher risk to acquire hookworm. Furthermore, *Giardia* assemblage D was reported in human (Broglia et al., 2013) as well as animal-specific assemblage, C and F (Soliman et al., 2011; Liu et al., 2014; Strkolcova et al., 2015) while assemblage D was not widely recognized as a zoonotic assemblage.

The limitation of this study was that we only used the remaining sediments from centrifugal sedimentation technique to further extract genomic DNA. Also, we were not able to amplify hookworm DNA from several samples, 40.1% (83/207) of hookworm-positive by conventional techniques hence making low hookworm-positive rate by PCR compared with another study (Ngui et al., 2012). Although we anticipated that concentrated samples obtained as sediments could yield higher amount of DNA than that from fresh fecal samples, storage of sediments in -20°C for 1-2 years may somewhat affect the quality of DNA. This in turn led to false negative by PCR despite the higher sensitivity of this technique in general. Long-term storage in -20°C of DNA samples might affect the protein conformation making the egg shell more difficult to be lysed using only heat thaw cycles before DNA extraction. However, mechanical treatments using glass bead and sonication as well as

examining for intact hookworm eggs after treatment to make sure that helminth eggs were successfully broken should be implemented. There were 20.7% (43/207) negative for both PCR amplifying hookworm gene and bacterial *16S rRNA* negative PCR results. These negative results may be caused by several factors including quality, quantity and contaminated PCR inhibitor including bilirubin, bile salts, heavy metals, hemoglobin degradation products and complex polysaccharides (Yao et al., 2018). Post DNA extraction procedures using products which can purify the DNA samples can improve DNA purity by eliminating any contaminants. However, to assess the effect of PCR inhibitors, spiking hookworm gene into negative samples and preceding with PCR can prove the presence of contaminants in DNA samples. Internal control, PCR targeting *16S* of bacterial DNA were implemented to verify the overall quality of DNA but was not directly related to the quality of helminth's genomic DNA because enteric bacteria were abundant in feces and DNA were easily extracted by DNA extraction procedures. The feline housekeeping gene, *NADH subunit 6*, is suggested for accessing a high PCR failure rate (Yao et al., 2018). However, bacterial *16s rRNA* has a higher amplifying rate compare with the feline *NADH* gene. Apart from that, the second PCR targeting short product of *ITS2* fragment of hookworm was tested. When product fragments from 2 PCRs were compared, the second PCR seems to be able to amplify shorter product than the first PCR thus making it supposed to be easier to amplify this product.

Acknowledgments

This research was partially funded to WS by The Chulalongkorn University Research Unit for Feline Infectious Disease and Health for Excellence, The Chulalongkorn University Research Unit for Microbial Food Safety and Antimicrobial Resistance, The Chulalongkorn University Research Unit (GRU 6203331007-1) and to WJ by The 100th Anniversary Chulalongkorn University for Doctoral Scholarship and The 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). The authors would like to gratefully thank Dr. Sahatchai Tangtrongsup for *Giardia* retesting from some samples.

Chapter 4

Unrevealing the neglected *Strongyloides* spp. infection in cats in Bangkok, Thailand.

Wanarit Jitsamai[a], Poom Adisakwattana[b], Woraporn Sukhumavasi[a,*]

[a]Parasitology Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University Bangkok 10330, Thailand

[b]Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

*Corresponding

E-mail address: vetkwan@hotmail.com

Keywords:

Cats, Hypervariable region, Scanning electron microscope, *Strongyloides*, Thailand

Abstract

Threadworms, *Strongyloides* spp., are soil-transmitted parasitic nematodes infecting a wide range host including human worldwide. Cats are infected by 4 species including *S. stercoralis*, a zoonotic species, *S. tumefaciens*, *S. planiceps* and *S. felis*. Only one report demonstrated the prevalence of threadworm in cats in Thailand and identified as *Strongyloides* spp. In order to identify cat threadweorm species, morphology, ultrastructure and molecular characterizing partial *18S rRNA* gene were conducted. From a total of 326 fecal samples, *Ancylostoma* spp. eggs are predominantly found 21.6% (128/326) and followed by *Toxocara* spp., *Platynosomum fastosum*, rhabditiform larvae, *Cystoisospora felis*, *Cystoisospora rivolta, Dipylidium caninum, Taenia taeniaeformis*, *Spirometra* spp., tapeworm eggs and *Eucoleus aerophilus* were 6.4% (21/326), 5.5% (18/326), 4.3% (14/326), 4.3% (14/326), 3.7% (12/326), 2.5% (8/326), 1.8% (6/326), 1.2% (4/326) 0.3% (1/326) and 0.3% (1/326), respectively. Rhabditifrom larvae were identified as *Strongyloides* and subjected to culture. Free-living adult were collected and observed under light microscope and scanning electron microscope. Post vulva constriction, key feature which found in *S. felis* and lacking in *S. stercoralis* was distinct and suggested that cat threadworm isolated from this study were *S. felis*. Ultrastructure of *en face* views revealed hexagonal stoma surrounded by circumorally elevations in both male and female. Characterizing partial *18S rRNA* including hypervariable region I demonstrated cat threadworm was distinguished from other species but still grouped in *S. stercoralis* and *S. procyonis* clade. Cat threadworm isolated from Bangkok supposed to be *S. felis***.** Complete *18S rRNA* gene including HVR I-IV analysis are suggested in order to confirm threadworm species.

Introduction

Threadworms, *Strongyloides* spp., are soil-transmitted parasitic nematodes infecting a wide range of domestic and wildlife animal species including human worldwide. Various species of *Strongyloides* have complex life cycles including free-living and parasitic stage. Only parasitic females cause pathogenicity while free-living stage is observed in environment. Free-living sexual life cycle can undergo more than one time depending on their species. The pattern of free-living life cycle in different species was adapted particularly with their host and environment combination which were selectively advantageous to maintain their fines (Dorris et al., 2002). Over 40 species of *Strongyloides* are parasitic nematode of vertebrate while the majority of host is mammal. Three species are reported infecting human. WHO reported that 30-100 million human infected with human threadworm, *Strongyloides stercoralis* which commonly found worldwide (Bethony et al., 2006). *S. fuelleborni* was attributed to two subspecies, *S. fuelleborni fuelleborni* and *S. fuelleborni kelleyi*. *S. fuelleborni fuelleborni* is reported in non-human primate and human in Africa while *S. fuelleborni kelleyi* infected human potentially causing fatal human strongyloidiasis which reported in New Guinea. These two subspecies are distinguished from morphology of parasitic females, free-living males and geographical distribution (Dorris et al., 2002).

S. stercoralis infected human, non-human primate and dogs worldwide. Cats can be infected by 4 species including *S. stercoralis*, a zoonotic species, *S. tumefaciens* (reported in North America and India), *S. planiceps* (reported in Japan and Malaysia) and *S. felis* (reported in India and Australia) (Thamsborg et al., 2017). The prevalence of *Strongyloides* in cats in Europe and Africa was not specified and potentially assumed as *S. stercolaris*. In 2019, *S. stercolaris* was firstly reported in natural infected cats causing colonic epithelial nodular hyperplasia (Wulcan et al., 2019). However, the lesions were morphologically similar with *S. tumefaciens* infected previously described and the morphology of *S. tumefaciens* is limited. Dogs have roles in zoonotic transmission cycle of *S. stercoralis* but, in cats, no evidence has been described. Major routes of transmission of *S. stercoralis* are percutaneous and mucosal penetration via oral route whereas the mode of infection for other

species in cats is not completely known. Most of cat threadworms reside in the small intestine except for *S. tumefaciens* that inhabits the large intestine nodules. Parasitic females produce larva stage 1 (L1) or partially embryonated eggs, only for *S. planiceps*, and shed in feces. Natural infection with *S. felis* may result in acute watery diarrhea but the experimental infection revealed that cats did not have any clinical signs with focal granulomas and subpleural inflammatory plaques associated with larval migration (Bowman et al., 2008). Although most *Strongyloides* infections in cats seem to be asymptomatic but potential zoonotic capacity has not yet been determined. So far, there is only one report that demonstrated the prevalence of threadworm in cats in Thailand (Rojekittikhun et al., 2014).

According to *Strongyloides* spp. infected wide range of domestic animal with host specificity. In the prevalence studies, S*trongyliodes* species are usually identified base on morphology and their host without genetic characterization. The validity of *Strongyloides* infected cat identification using both morphology and genetic identification need to be reappraised.

Materials and Methods

Study design, sample collection and examination

The cross-sectional study was constructed to collect fecal samples from veterinary neutering services unit, the Center for Animal Reservoir Control, Department of Livestock Development. The project was approved by Chulalongkorn University Institutional Animal Care and Use Committee (Animal Use Protocol No. 1631039). Feces were collected by rectum and/or colon flushing using 10 ml sterile normal saline via feeding tube. PBS-ethyl acetate centrifugal sedimentation and/or $ZnSO_4$ centrifugal flotation (specific gravity 1.18) (Dryden et al., 2005) were used in order to detected enteric parasites.

Larva identification and modified agar plate culture

Larva were identified under the light microscope using *Strongyloides* characteristics including short buccal cavity, rhabditiform esophagus, straight tail and a prominent genital primordium. Agar media composed of 1.5% agar, 0.5% meat

extract, 1.0% peptone, and 0.5% NaCl was prepared (Koga et al., 1991; Khanna et al., 2015). Approximately 2g of *Strongyloides* larva-positive feces was placed on the agar and were observed under the stereomicroscope every 24 hours. Free-living adults and larvae were collected in 2.5% glutaraldehyde and lysis buffer to preserve for further analysis.

Scanning electron microscope

Samples preserved in 2.5% glutaraldehyde were rinsed twice with 0.1 M phosphate buffer pH 7.2 and once with distilled water for 10 minutes each. Samples were dehydrated with a graded series of ethanol (30%, 50%, 70%, 95% and 100% for 10 minutes/time). Critical point dry (critical point dry, Quorum model K850, UK), mounting and coating with gold (sputter coater, Balzers model SCD 040, Germany) were performed. Coated samples were observed under scanning electron microscope (JEOL, model JSM 0010LV, Japan).

Molecular identification

The species of *Strongyloides* were identified using conventional polymerase chain reaction by targeting the fragment of *18S rRNA* gene followed by sequencing. Due to the lack of *Strongyloides* in cats in DNA sequence database, various universal primers for *Strongyloides* and its relative were used. Larvae and adults recovered from modified agar plate culture were subjected to DNA extraction using QIAamp DNA Stool Mini Kit (Qiagen, USA) according manufacturer's protocol with three cycles of freeze (one minute in liquid nitrogen) and thaw (five minutes boiling in water bath). Fragment of *18S rRNA* gene were amplified by using 2 separate set primer including (i) SS18sF (5'-TATGCATCTTCTCGGAAACGA-3') and SS18sR (5'-CATAAAAGTCTCGCTG TTATC-3') (ii) Nem18SF 5'-CGCGAATRGCTCATTACAAC AGC-3' and Nem18SR (5'-GGGCGGTATCTGATCGCC-3'). The reactions of SS18sF and SS18sR were performed for 30 cycles of 94°C for 30s, 53°C for 30s, and 72°C for 30s with an initial denaturation (94°C for 3 min) and a final extension (72°C for 10 min) (Floyd et al., 2005). The reactions of Nem18SF and Nem18SR were performed for 35 cycles of 94°C for 30s, 54°C for 30s, and 72°C for 1 min with an initial denaturation (94°C for 5

min) and a final extension (72°C for 10 min). The PCR products were analyzed by 1.5% agarose gel electrophoresis with SYBR Safe DNA Gel Stain and visualized under ultraviolet transilluminator followed by sequencing. Fragments of *18S RNA* were aligned and analyzed the four hyper-variable regions (HVR) and compared with other *Strongyloides* species.

Results

Prevalence of enteric parasites and demographic data of *Strongyloides*-infected cats.

From a total of 326 fecal samples, *Ancylostoma* spp. eggs are predominantly found 39.3% (128/326) and followed by *Toxocara* spp., *Platynosomum fastosum*, rhabditiform larvae, *Cystoisospora felis*, *Cystoisospora rivolta, Dipylidium caninum, Taenia taeniaeformis*, *Spirometra* spp., tapeworm eggs and *Eucoleus aerophilus* were 6.4% (21/326), 5.5% (18/326), 4.3% (14/326), 4.3% (14/326), 3.7% (12/326), 2.5% (8/326), 1.8% (6/326), 1.2% (4/326) 0.3% (1/326) and 0.3% (1/326), respectively. All rhabditiform larvae infected cats were identified as *Strongyloides* spp. infection based on their larval characteristics. The demographic data of *Strongyloides* infected cats are shown in Table 10. All 14 *Strongyloides*-infected cats were detected by PBS-ethyl acetate centrifugal sedimentation. Only 6 samples out of 14 were tested with $ZnSO_4$ centrifugal flotation and found *Strongyloides* 50.0% (3/6). *Ancylostoma* spp. or hookworm was predominantly found coinfection with *Strongyloides*, 28.6% (4/14) followed by single infection 14.3% (2/14), with hookworm and *P. fastosum* 14.3% (2/14), with hookworm and *C. rivolta* 7.1% (1/14), with hookworm, *P. fastosum* and *C. rivolta* 7.1% (1/14), with hookworm and *T. taeniaeformis* 7.1% (1/14), with hookworm and *Spirometra* spp. 7.1% (1/14) and with *Toxocara* spp. 7.1% (1/14).

Table 10 Demographic data showing signalment and history of cats infecting with threadworm (n = 14)

Demographic data	% (n)
Sex	
Male	85.7 (12)
Female	14.3 (2)
Age	
≤1 year	14.3 (2)
1-3 years	35.7 (5)
>3 years	7.1 (1)
Ownership	
Owned	42.9 (6)
Stray	57.1 (8)
Ability to access outdoors	
Outdoors	71.4 (10)
Strictly indoors	28.6 (4)
Multi-cat household	(6)

Morphology of *Strongyloides* larvae collecting from cats

The features which used to identify *Strongyloides* larvae included a prominent genital primordium, rhabditiform oesophagus and straight narrowing tail. The average size was 243.5 ± 28.8 µm in length and 20.3 ± 6.8 µm in width.

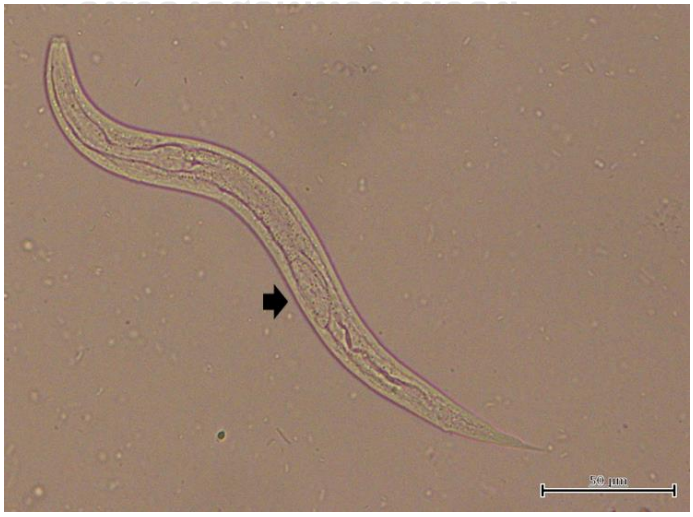


Figure 6 Morphology under light microscope of rhabditiform larva. Prominent genital primordium is demonstrated by arrow head.

Morphology of free-living males and females of *Strongyloides* spp. adults collecting from cats

A total 6 out of 14 threadworm positive samples were subjected to culture using modified agar plate culture. A free-living male and female adult of *Strongyloides* spp. recovered 54 hours post fecal collection was demonstrated in figure 2. The average length of free-living males and females was 745.8 ± 116.1 µm and 1229.9 ± 126.2 µm, respectively. Post vulva constriction was distinct in female adults.

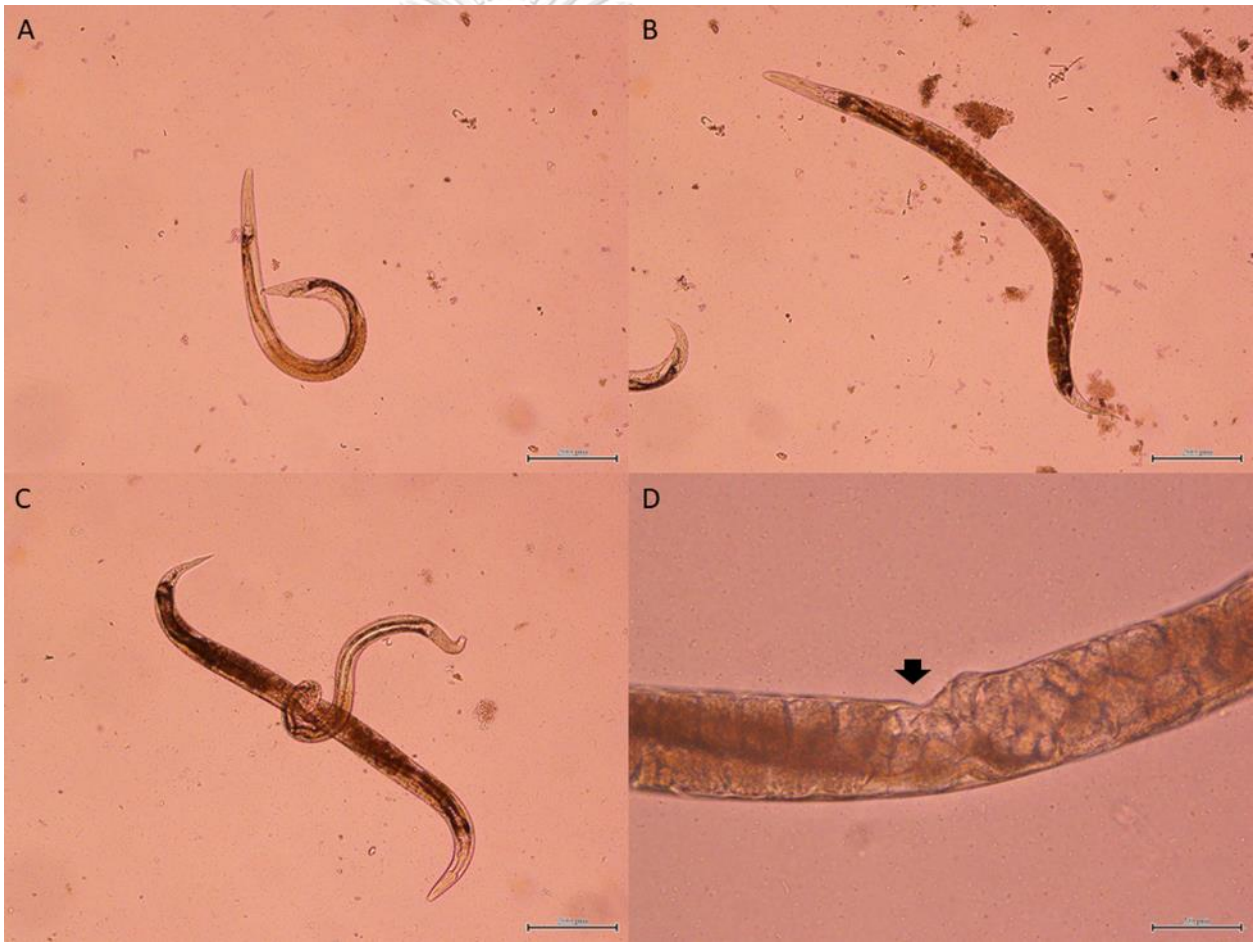


Figure 7 Morphology under light microscope of free-living male (A) and female (B). Courtship behavior was observed 54 hours post fecal collection (C). Post-vulva constriction (D) is demonstrated by arrow head.

Ultrastructure of free-living males and females of *Strongyloides* spp. adults collecting from cats

The *en face* views of free living female revealed a hexagonal stoma surrounded by the two level stacking circumorally elevations with six lobes each at the cephalic extremity. Post vulva constriction was distinct. The anus to tail distance is 77.9 ± 3.3 µm. In free living male, the *en face* views revealed a hexagonal stoma surrounded by the two level stacking circumorally elevation with six lobes each at the cephalic extremity as well as in female. At terminal end, four pairs of papillae were seen including 2 pairs at the cloaca level on the ventral side, 1 pair on ventral sub terminal end of tail and 1 pair on dorsal sub terminal end of tail. One pair of spicules associated with gubernaculum was observed.

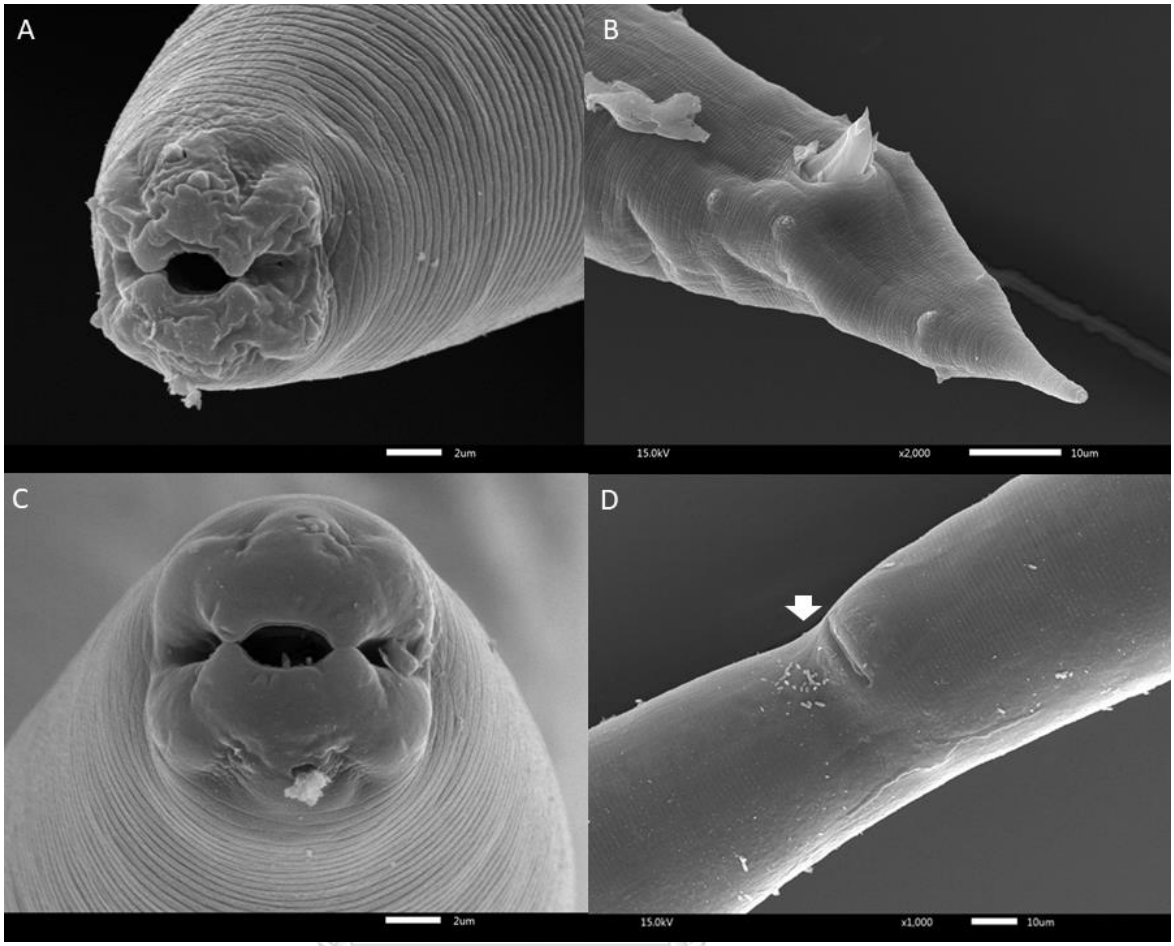


Figure 8 Ultrastructure of free-living male including cephalic end (A) and tail end (B) are demonstrated. The cephalic end of free-living female (C) is shown and post-vulva constriction (D) is demonstrated by arrow head.

Genetic characterization of *Strongyloides* collecting from cats

Three separate fragments including 252, 197, 259 base pairs were obtained from PCR targeting *18S rRNA* gene. All fragments were combined and constructed phylogenetic tree using Tamura 3-parameter model. Various *Strongyloides* species were included in phylogram using *Parastrongyloides trichosuri* and *Rhabditophanes* spp. as out groups. Phylogenetic tree is shown in figure 9. Cat *Strongyloide*s is distinguished from other species. However, cat *Strongyloides* still grouped in *S. stercoralis* and *S. procyonis* clade. HVR-I analysis from various species of *Strongyloides* were showed in Figure 10. Base on HVR-I, *S. papillosus*, *S. venezuelensis* and *S. ratti* share the same arrangement of nucleotide. *S. procyonis*,

three isolates of *S. stercoralis* and cat *Strongyloides* differed only in the number of T repetitive.

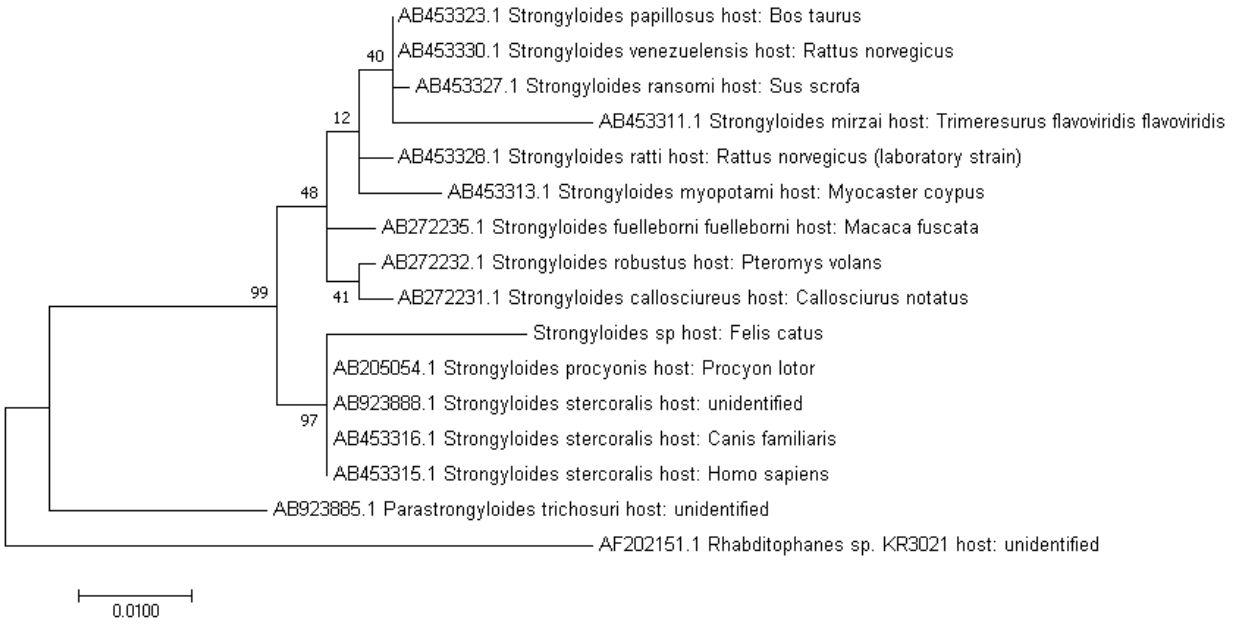


Figure 9 Phylogenetic tree demonstrate cat *Strongyloides* with various species of *Strongyloides*

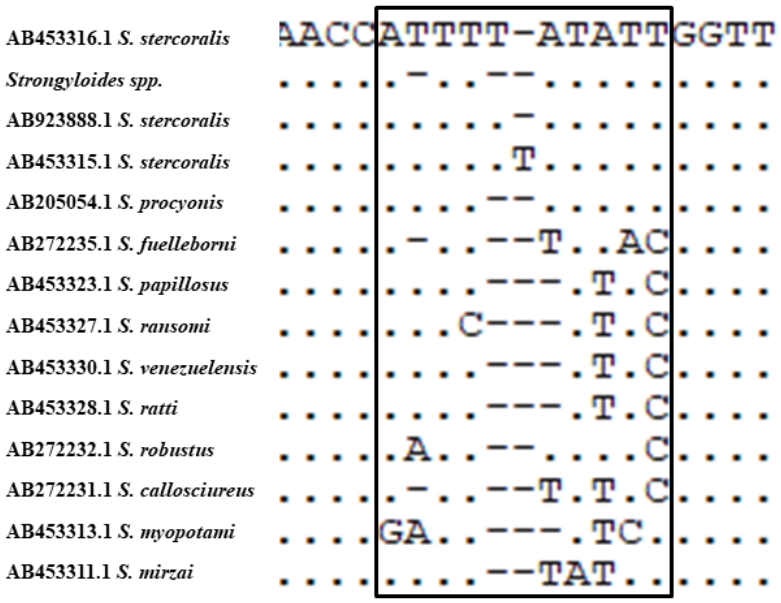


Figure 10 Nucleotide arrangements in *HVR-I* (box) in *18S rRNA* gene of *Strongyloides* spp.; dot indicates homologous with *S. stercoralis* (AB453316.1); dash indicates absence of nucleotide

Discussions

The prevalence of enteric parasites in cats this study has similar trend with the prevalence collecting in 2014-2015 from Bangkok and vicinity. Interestingly, rhabditiform larvae were found only in present study, 2016 by both sedimentation and flotation although in centrifugal flotation, larvae appearances were distorted due to unappropriated osmolality from $ZnSO_4$. However, Baermann technique is recommended in order to increase larvae detection rate (Speare and Tinsley, 1986; Bowman et al., 2008). Hookworm was the most commonly found coinfection with rhabditiform larvae. Hookworm is the most common enteric parasite and can making threadworm become opportunistic infection. Moreover, coinfection of hookworm and threadworm in old feces may result in misdiagnosis due to hookworm can develop and also yield rhabditiform larvae. Apart from larvae morphology, freshly collected feces can help to differentiate threadworm larvae from hookworm larvae.

According to fecal samples were directly collected from cats and tested within the same day, hookworm larvae were excluded from differential list and prominent genital primordium was obviously seen in living larvae thus making it is clear to identify as *Strongyloides* spp. Cats can be naturally infected by 4 species including *S. stercoralis*, *S. tumefaciens*, *S. planiceps* and *S. felis*. We excluded *S. planiceps* due to rhabditiform larvae were found while *S. planiceps* yield larvated eggs to feces. The body of free-living females in this study was constricted immediately posterior to vulva which is in line with *S. felis* and lacking in *S. stercoralis* free-living females (Bowman et al., 2008). *Strongyloides* in this study supposed to be *S. felis*. The identification features of *S. felis* included (i) a medium size of parasitic females inhabit in proximal part of small intestine, (ii) with hair pin type ovary, (iii) narrow tapered tail, (iv) larvae seen in fresh feces and (v) free-living females with post vulva constriction (Speare and Tinsley, 1986). Only two features (iv, v) were demonstrated in present study due to parasitic females were not collected.

Free-living adult cat *Strongyloides* produced eggs that hatched to larvae and developed to infective filariform larvae taken approximately 6 days at 20°c (Speare and Tinsley, 1986). In present study, rhabditiform larvae were taken about 2 days post fecal collection to become free-living adult and filariform larvae were observed

4 - 5 days post fecal collection. Fecal culture in room temperature 25 - 30°c in present study might decrease time for developing to infective stage. However, we defined hookworm filariform larvae using criteria including filariform esophagus occupied 2/3 of total length with point tail while *S. felis* esophagus occupied 1/3 of total length with truncated tail which end in a number of points (Speare and Tinsley, 1986). Free-living adult of *S. felis* had a short life and only one generation was occurred. Almost infected cats were raise outdoors that support with threadworm route of transmission. Interestingly, threadworm are found in cat which age less than 1 year which contrast with *S. felis* was found almost exclusive in older cats and transmammary was not reported thus making this parasite was not commonly found in kittens (Bowman et al., 2008). Experimental infection with S. *felis* was persisted over 2 years (Speare and Tinsley, 1986).

We firstly reported ultrastructure of free living adult *S felis* including *en face* view, post vulva constriction and tail. The *en face view* especially in parasitic females was proved to be the most useful in order to identify *Strongyloides* (Little, 1966; Sato et al., 2008). Shape of stoma and number of lobes on the circumoral elevation were considered as the important diagnostic features. However, examination of minute nematodes like *Strongyloides* under light microscope with oil immersion is difficult. The identified features are usually not clear, hard to interpret and need more experience to diagnosis (Little, 1966). Base on parasitic females, stoma shape were classified into the angular stoma (*S. procyonis* and *S. martis*), complex stoma (*S. robustus*, *S. callosciureus*, *S. planiceps*, *S. papillosus* and *S. myopotami*), and stoma with esophageal teeth (*S. ransomi*) (Sato et al., 2008). Hexagonal stoma with circumoral elevation found in free-living female cat *Strongyloides* also found in *S. procyonis* and *S. stercoralis* parasitic female. However, we cannot directly compare stoma morphology between free-living and parasitic stage. This study also provided ultrastructure of post vulva constriction to support morphological finding using light microscope.

Molecular characterization of partial *18S rRNA* gene revealed cat *Strongyloides* is genetically distinct from other *Strongyloides* but still grouped in *S. stercoralis* and *S. procyonis* clade. Previous study described that *S. stercoralis* and *S.*

felis shared morphologically features (Chandler, 1925; Speare and Tinsley, 1986) which result in given scientific nomenclature as *S. stercoralis felis* (Chandler, 1925). Due to highly conserved regions of *18S rRNA* gene in *Strongyloides* species, *18S rRNA* gene was not suitable for species identification (Hasegawa et al., 2009). However, small fragments containing short stem and loop in *18S rRNA* gene or hyper-variable regions I–IV (HVR I -IV) are more variable in nucleotide arrangement among species and could be able to identify *Strongyloides* species. This study provided nucleotide arrangement of HVR-I which is comprised of 8-11 nucleotides. HVR-1 sequence revealed that cat threadworm seems to be more similar with *S. stercoralis* and *S. procyonis* related with phylogenetric tree analysis of partial *18S rRNA* gene. However, HVR-IV containing 23-39 nucleotide is longer than other HVR and suitable for species identification because it nucleotide arrangement are mostly species specific (Hasegawa et al., 2009). To differentiated *S. felis* from *S. stercoralis*, HVR-II to IV analysis is suggested.

S. felis was firstly discovered in 1925 in domestic cats living in India at the prevalence 20% (Chandler, 1925). Chandler considered that cat threadworm was grouped with *S. stercoralis* and also proposed that cat threadworm should name *S. stercoralis felis* according to morphologically similar with *S. stercoralis*. *S. felis* was also reported again 61 years later in Australia (Speare and Tinsley, 1986; Speare and Tinsley, 1987). Pathological changes during infection were found in small intestine and lung. Parasitic females were found in mucosa of the crypts of upper small intestine with no inflammatory response. Some infections were associated with adenomatous metaplasia of glandular epithelium. Infective larvae migrated to lung causing hemorrhage in alveolar and interalveolar septa. Clinical appearances of *S. felis* infection were not consistent. Diarrhea was not major clinical feature but in heavy infection, acute watery diarrhea might be seen (Speare and Tinsley, 1986).

This study provided preliminary information of cat threadworm, *S. felis*. Apart from morphology and ultrastructure of free living adult, to clearly identify *S. felis*, cat threadworm parasitic female should be examined both morphology under microscope and ultrastructure as well as complete *18S rRNA* gene including HVR I-IV should be characterized

Acknowledgements

This research was partially funded to WS by The Chulalongkorn University Research Unit for Feline Infectious Disease and Health for Excellence, The Chulalongkorn University Research Unit for Microbial Food Safety and Antimicrobial Resistance, The Chulalongkorn University Research Unit (GRU 6203331007-1) and to WJ by The 100th Anniversary Chulalongkorn University for Doctoral Scholarship and The 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). Moreover, authors would like to thank undergraduate student volunteers (Pluemhathaikij L., Harncharoenkul P., Powjinda T., Pornlumfah P.) and graduate students in parasitology unit, Faculty of Veterinary Science, Chulalongkorn University for cooperating in sample collection and processing.

Chapter 5

General discussion, conclusion, and further recommendations

This study provided the prevalence of endoparasites in cats living in Bangkok and vicinity collected during 2014-2015 and in 2016. The prevalence is shown in Table. The prevalence tends to be similar which hookworm, *Ancylostoma* spp. was the predominantly found. Interestingly, comparing with the prevalence of endoparasites in cats in Bangkok, 2005 reveals that the prevalence seems to be higher than previous (Jittapalapong et al., 2007a). While anti-helminthic products were developed, the prevalence of endoparasites was still high. Hence, client-owned cats could acquire infective stages as well as stray cats that share roaming area. The awareness of endoparasites prevention should be emphasized to the owners. Despite the absence of cyst and trophozoite by conventional method, this may be due to high sensitivity of the commercial test to potentially identify asymptomatic or reservoir cats. Since *Giardia* cysts are intermittent shed, the recommendation suggested that feces should be collected at least three times separately over 3 to 5 days and detected using $ZnSO_4$ centrifugal flotation (Vasilopulos et al., 2006).

Table 11 Prevalence of endoparasites in cats living in Bangkok and vicinities

Year	n	Status	Nematodes				Cestodes			Flukes		Protozoa	
			ANC	TOX	STR	EA	SPI	TT	DC	OPI	PF	CYS	Reference
2014-2015	509	Client-owned	21.6 (110)	6.9 (35)			1.6 (8)	2.9 (15)	0.4 (2)	0.2 (1)	3.7 (19)	3.5 (18)	Current study
2016	326	Client-owned	39.3 (128)	6.4 (21)	4.3 (14)	0.3 (1)	1.2 (4)	1.8 (6)	2.8 (9)		5.5 (18)	7.0 (26)	Current study
2005	1485	Stray	9.9 (148)	3.5 (52)		0.1 (1)		0.1 (1)	0.1 (2)		0.1 (1)	1.0 (15)	Jittapalapong et al., 2007

ANC; *Ancylostoma* spp., TOX; *Toxocara* spp., STR; *Strongyloides* spp., EA; *Eucoleus aerophilus*, SPI; *Spirometra* spp., TT; *Taenia taeniaeformis*, TAP; tapeworm eggs, DC; *Dipylidium caninum*, OPI; *Opisthorchis* spp., PF*; Platynosomum fastosum*, CYS; *Cystoisospora* spp.,

Soil-transmitted helminths including hookworm and roundworm were commonly detected which related to more than half of cats in this study could access outdoors and can acquired infective stages from contaminated soils.

Univariate analysis and multiple logistics regression revealed that significant factors associated with hookworm infection which related with its mode of transmission. The ability to access outdoors seems to be the major cause that cats can acquire infective stage of hookworm. In this population, the monthly deworming was found only in 9.3% of cats making the infection rate of endoparasites was quite high. These data imply that suitable parasitic prevention programs need to be emphasized to the cat owners. This study also reported foodborne helminths including *P. fastosum*, *D. caninum*, *T. taeniaeformis*, *Spirometra* spp., and *Opisthorchis*-like egg supporting that free-roaming cat but client-owned might have the possibility to be infected with these parasites by ingesting infective stages in intermediated hosts that might be found in their roaming area.

Almost previous studies revealed that *Ancylostoma* spp. was almost always a predominant enteric parasite in Thailand with prevalence 3.0 - 77.3% and also commonly found in Malaysia and Indonesia with prevalence 37.9 - 78.0% (Margono et al., 1979a; Ngui et al., 2014). Molecular characterization base on partial *ITS1, 5.8S* and *ITS2* in this study demonstrated that almost cat hookworm in Bangkok and vicinity are *A. ceylanicum* which supported a survey conducted in the lower northern part of Thailand (Pumidonming et al., 2017) and morphological survey several years ago (Setasuban et al., 1976). Hookworm is worldwide distributed but not imperfectly due to overlapping climate range. *A. braziliense* is found along south Atlantic coastal regions of North America, the Caribbean Sea and South America. *A. caninum* and *A. tubaformae* are found in warmer areas (Bowman et al., 2010) while *A. ceylanicum* is found in Australia and Asia including China, Laos, Malaysia, Taiwan and Thailand. *A. ceylanicum* is a predominant cat hookworm in Southeast Asia and considered as a neglected zoonosis disease. Although *A. ceylanicum* tends not to be a causative agent of cutaneous larvae migrants (Bowman et al., 2010), it occasionally develops into adults in human causing abdominal discomfort. High prevalence of *A. ceylanicum* infected dogs and cats in China was considered as a risk factor associated with human infection (Chen et al., 2012). Hence, dogs have a role of reservoirs for the zoonotic transmission of *A. ceylanicum* (Conlan et al., 2012; Inpankaew et al., 2014a; Thompson, 2015) but the role of cats has not been addressed yet.

Characterized of *COX1* gene of cat hookworm revealed that cat hookworm tends to be distinct from human hookworm. This study also firstly reported *A. tubaeforme* in Thailand. *A. tubaeforme* is widely distributed including Australia, Brazil, Italy, Qatar, Spain, the United States of America and China (Fu et al., 2019b). Heavy *A. tubaeforme* infection results in decreasing pack cell volume and hemoglobin rapidly causing anemia suggesting that the hookworm positive cats should not be neglected.

Cats become infected with *Giardia* assemblage A, B and F (Thompson and Ash, 2016). The previous study revealed that the majority of *Giardia* spp. from stray dogs in Thai temples in Bangkok was assemblage A (Inpankaew et al., 2007). In cats, we surprisingly found that only one sample which positive is *Giardia* assemblage D which commonly found in dogs. However, assemblage D was also found in cats in Chiang Mai, Thailand (Tangtrongsup et al., 2020), Japan and Australia (Palmer et al., 2008; Ito et al., 2017). We hypothesized that cats potentially acquired assemblage D from dogs. Hence, *Giardia* assemblage D was reported in human (Broglia et al., 2013) However, assemblage D was not widely recognized as a zoonotic assemblage.

Interestingly, rhabditiform larvae were found only in 2016. Prominent genital primordium was obviously seen in living larvae thus making it is clear to identify as *Strongyloides* spp. Cats can be naturally infected by 4 species including *S. stercoralis*, *S. tumefaciens*, *S. planiceps* and *S. felis*. The body of free-living females in this study was constricted immediately posterior to vulva which is in line with *S. felis* and lacking in *S. stercoralis* free-living females (Bowman et al., 2008). *Strongyloides* in this study supposed to be *S. felis*. However, we cannot definitively identify due to parasitic females were not observed. In agar plate culture, rhabditiform larvae were taken about 2 days post fecal collection to become free-living adult and filariform larvae were observed 4-5 days post fecal collection. Almost threadworm infected cats were raised outdoors supporting threadworm route of transmission. Interestingly, threadworm are found in cat which age less than 1 year inconsistent with *S. felis* was almost exclusively found in older cats and transmammary was not reported thus making this parasite was not commonly found in kittens (Bowman et al., 2008). We also firstly provided ultrastructure including *en face* view, post vulva constriction and tail of free living adult cat threadworm. The *en face view* of parasitic

females was useful to identify *Strongyloides* levels (Little, 1966; Sato et al., 2008). Base on parasitic females, stoma shape were classified into the angular stoma complex stoma and stoma with esophageal teeth (Sato et al., 2008). Hexagonal stoma with circumoral elevation found in free-living female cat *Strongyloides* also found in *S. procyonis* and *S. stercoralis* parasitic female. However, we cannot directly compared stoma morphology between free-living and parasitic stage.

Molecular characterization of partial *18S rRNA* gene revealed cat *Strongyloides* was genetically distinct from other *Strongyloides* but still grouped with *S. stercoralis* and *S. procyonis* clade., *18S rRNA* gene was not suitable for identification in species level due to it highly conserved in *Strongyloides* species (Hasegawa et al., 2009). However, hyper-variable regions I–IV (HVR I -IV) are more variable in nucleotide arrangement and could be able to identify *Strongyloides* species. Nucleotide arrangement of HVR-I which is comprised of 8-11 nucleotides was analyzed. HVR-1 revealed that cat threadworm seems to be more similar with *S. stercoralis* and *S. procyonis* related with analysis of partial *18S rRNA* gene. However, HVR-IV containing 23-39 nucleotide is suitable for species identification because its nucleotide arrangement are mostly species specific (Hasegawa et al., 2009). To differentiated *S. felis* from *S. stercoralis*, HVR-II to IV analysis is suggested.

For the limitation, since cat population from our study excluded cats below 6 months of age due to reproductively immature for neutering, prevalence of parasites commonly found in kitten maybe underestimated including *Toxocara* spp., *Cystoisospora* spp. and *Giardia* spp. Further study to include kitten population will help to reflect overall parasitic problems in cats especially zoonotic concerns of *Toxocara* spp., *Toxoplasma gondii* and *Giardia*. According to feces were directly collected from cats during anesthesia thus making the amount of feces were limited. Before neutering, cats need to be fasting to avoid aspiration pneumonia from vomiting before or after anesthesia, so cats have less amount of fecal content remained in their rectum, the quantity of feces was not enough for all fecal examinations, agar plate culture and molecular diagnosis. Furthermore, previous study suggested that fresh feces are suitable for DNA extraction. PCR detecting parasitic eggs in feces are challenging due to contaminated PCR inhibitor in feces

including bilirubin, bile salts, heavy metals, hemoglobin degradation products and complex polysaccharides which might affected to PCR reaction (Yao et al., 2018). We also suggested that internal control such as PCR targeting bacterial *16S rRNA* gene or feline housekeeping gene, *NADH subunit 6* should be implemented to verify false negative results.

This study provided advantages to various people including cat owners, veterinarians, veterinary students, medical and public health staffs and scientists. The prevalence from of this study will help to define the role of cats as reservoirs for enteric helminths and protozoa in Bangkok and vicinity, Thailand especially for hookworm, threadworm and *Giardia* spp. This study suggested that cat hookworm and *Giardia* spp. have less potential of zoonotic transmission. However, potential zoonotic capacity of cat threadworm has not yet been determined. Risk factors associated with hookworm infection in cats will be beneficial to educate both clients and veterinarian for awareness in zoonotic potentials. Furthermore, methods from this study can be applied in order to detect enteric helminths and protozoa from cats.

Appendix 1

แบบฟอร์มข้อมูลตัวอย่างเลือดและอุจจาระจากสุนัขและแมวที่มาเข้ารับบริการ
ณ หน่วยรักษาสัตว์เลี้ยงเคลื่อนที่ โครงการสัตวแพทย์อาสาเฉลิมพระเกียรติ 60 พรรษา
สมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี

LabID……..…

ตัวอย่างที่เก็บ ☐ เลือด ☐ อุจจาระ ล้วง / flush / เก็บจากพื้น

เจ้าของกรณากรอกประวัติส่วนนี้ก่อน

ชื่อเจ้าของ ……..……โทรศัพท์มือถือ.....โทรศัพท์บ้าน.....

ที่อยู่ของสัตว์.....

ชื่อสัตว์ ……..…….....ชนิด ☐ สุนัข ☐ แมว พันธุ์……..…เพศ ☐ ผู้ ☐ ผู้ทำหมัน ☐ เมีย ☐ เมียทำหมัน

อายุ……น้ำหนัก.....

การเลี้ยง ☐ ภายในตัวบ้าน ☐ นอกตัวบ้านแต่อยู่ในรั้ว ☐ นอกรั้วบ้าน

อาหาร ☐ อาหารทำเอง ☐ อาหารสำเร็จรูป ☐ ผสมระหว่างอาหารทำเองกับอาหารสำเร็จรูป

สัตวเลี้ยงอื่นๆในบ้าน ☐ ไม่มี ☐ สุนัข……ตัว ☐ แมว……ตัว ☐ อื่นๆ……..…

โอกาสสัมผัสกับสัตว์อื่นนอกบ้าน เช่น สัตว์ของเพื่อนบ้าน สัตว์จรจัด ☐ ไม่มี ☐ สุนัข ☐ แมว ☐ อื่นๆ……

การวัคซีน ☐ ไม่ทำเลย ☐ วัคซีนรวม ☐ วัคซีนพิษสุนัขบ้า ☐ วัคซีนอื่นๆ..... ☐ ไม่ทราบ

การกระตุ้นวัคซีน ☐ ไม่เคยกระตุ้น ☐ ประจำทุกปี ☐ ไม่ประจำทุกปี.....…. ☐ ไม่ทราบ

การถ่ายพยาธิ ☐ ไม่เคยทำ ☐ ประจำทุกเดือน ☐ ไม่ประจำทุกเดือ แต่ทำทุก.....เดือน ☐ ไม่ทราบ

การป้องกันพยาธิหนอนหัวใจ ☐ ไม่เคยทำ ☐ ประจำทุกเดือน ☐ ไม่ประจำทุกเดือน……☐ ไม่ทราบ

การป้องกันเห็บหมัด ☐ ไม่เคยทำ ☐ ประจำทุกเดือน ☐ ไม่ประจำทุกเดือน ☐ ไม่ทราบ

เคยตรวจพบว่าเป็นโรค (ด้วยวิธี)

☐ ไม่เคยตรวจ ☐ ไม่เคยป่วย ☐ พยาธิเม็ดเลือด..... ☐ พยาธิหนอนหัวใจ.....

☐ ลิวคีเมียแมว…… ☐ เอดส์แมว……. ☐ อื่นๆ……

ข้อมูลอื่นๆ

1.มีเห็บตามตัวหรือไม่ ☐ พบ ☐ ไม่พบ ☐ ไม่ทราบ

2.มีหมัดตามตัวหรือไม่ ☐ พบ ☐ ไม่พบ ☐ ไม่พบตัวแต่พบขี้หมัด ☐ ไม่ทราบ

3.สัตว์มีโอกาสถูกยุงกัดหรือไม่ ☐ มี ☐ ไม่มี ☐ ไม่ทราบ

4.สัตว์เคยมีประวัติเดินทางไปต่างประเทศหรือไม่(เมื่อไหร่) ☐ เคย……. ☐ ไม่เคย

5.สัตว์เคยท้องเสียหรือไม่(เมื่อไหร่) ☐ เคย…….. ☐ ไม่เคย

6.เคยพบปล้องของพยาธิในอุจจาระหรือไม่ ☐ พบ ☐ ไม่พบ ☐ ไม่ทราบ

7.สัตว์เคยอาเจียนหรือไม่(เมื่อไหร่) ☐ เคย…….. ☐ ไม่เคย

8.เคยมีพฤติกรรมไล่จับสัตว์อื่นหรือไม่ ☐ ไม่เคย ☐ เคย

ถ้าเคย ☐ หนู ☐ สัตว์เลื้อยคลาน ชนิด ..... ☐ ปลา ☐ กบ ☐ แมลง ชนิด ……. ☐ อื่นๆ……

9.สัตว์เคยดื่มน้ำในแหล่งน้ำตามธรรมชาติ ☐ เคย ☐ ไม่เคย

Appendix 2

<u>แบบฟอร์มข้อมูลตัวอย่างเลือดและอุจจาระจากสุนัขและแมวที่มาเข้ารับบริการ</u>
<u>ณ หน่วยรักษาสัตว์เลี้ยงเคลื่อนที่ โครงการสัตวแพทย์อาสาเฉลิมพระเกียรติ 60 พรรษา</u>
<u>สมเด็จพระเทพรัตนราชสุดาฯ สยามบรมราชกุมารี</u>

LabID.....

ตัวอย่างที่เก็บ ☐ เลือด ☐ อุจจาระ ล้วง / flush / เก็บจากพื้น

ชื่อเจ้าของ .....จำนวนสัตว์ทั้งหมดที่นำมารับบริการ.....
เบอร์โทรศัพท์มือถือ.....เบอร์โทรศัพท์บ้าน.....
ที่อยู่ของสัตว์.....

1.ชื่อสัตว์ ..... ☐ สุนัข☐ แมว พันธุ์..... อายุ.....เพศ ☐ ผู้☐ ผู้ทำหมัน☐ เมีย ☐ เมียทำหมัน
2.ชื่อสัตว์ ..... ☐ สุนัข☐ แมว พันธุ์..... อายุ.....เพศ ☐ ผู้☐ ผู้ทำหมัน☐ เมีย ☐ เมียทำหมัน
3.ชื่อสัตว์ ..... ☐ สุนัข☐ แมว พันธุ์..... อายุ.....เพศ ☐ ผู้☐ ผู้ทำหมัน☐ เมีย ☐ เมียทำหมัน
4.ชื่อสัตว์ ..... ☐ สุนัข☐ แมว พันธุ์..... อายุ.....เพศ ☐ ผู้☐ ผู้ทำหมัน☐ เมีย ☐ เมียทำหมัน
5.ชื่อสัตว์ ..... ☐ สุนัข☐ แมว พันธุ์..... อายุ.....เพศ ☐ ผู้☐ ผู้ทำหมัน☐ เมีย ☐ เมียทำหมัน
6.ชื่อสัตว์ ..... ☐ สุนัข☐ แมว พันธุ์..... อายุ.....เพศ ☐ ผู้☐ ผู้ทำหมัน☐ เมีย ☐ เมียทำหมัน

<u>การเลี้ยง</u> ☐ ภายในตัวบ้าน ☐ นอกตัวบ้านแต่อยู่ในรั้ว ☐ นอกรั้วบ้าน
<u>อาหาร</u> ☐ อาหารสำเร็จรูป ☐ อาหารทำเอง ☐ ผสมระหว่างอาหารทำเองกับอาหารสำเร็จรูป
<u>สัตว์เลี้ยงอื่นๆในบ้าน</u> ☐ ไม่มี ☐ สุนัข.....ตัว ☐ แมว.....ตัว ☐ อื่นๆ.....
<u>โอกาสสัมผัสกับสัตว์อื่นนอกบ้าน</u> เช่น สัตว์ของเพื่อนบ้าน สัตว์จรจัด ☐ ไม่มี ☐ สุนัข ☐ แมว ☐ อื่นๆ.....
<u>การวัคซีน</u> ☐ วัคซีนรวม ☐ วัคซีนพิษสุนัขบ้า ☐ วัคซีนอื่นๆ..... ☐ ไม่ทำเลย ☐ ไม่ทราบ
<u>การกระตุ้นวัคซีน</u> ☐ ประจำทุกปี ☐ ไม่ประจำทุกปี..... ☐ ไม่เคยกระตุ้น ☐ ไม่ทราบ
<u>การถ่ายพยาธิ</u> ☐ ประจำทุกเดือน ☐ ไม่ประจำทุกเดือ แต่ทำทุก.....เดือน ☐ ไม่เคยทำ ☐ ไม่ทราบ
<u>การป้องกันพยาธิหนอนหัวใจ</u> ☐ ประจำทุกเดือน ☐ ไม่ประจำทุกเดือน.....☐ ไม่เคยทำ ☐ ไม่ทราบ
<u>การป้องกันเห็บหมัด</u> ☐ ประจำทุกเดือน ☐ ไม่ประจำทุกเดือน ☐ ไม่เคยทำ ☐ ไม่ทราบ
<u>เคยตรวจพบว่าเป็นโรค (ด้วยวิธี)</u>
☐ ไม่เคยตรวจ ☐ ไม่เคยป่วย ☐ พยาธิเม็ดเลือด..... ☐ พยาธิหนอนหัวใจ.....
☐ ลิวคีเมียแมว..... ☐ เอดส์แมว..... ☐ อื่นๆ.....
<u>ข้อมูลอื่นๆ</u>
1.มีเห็บตามตัวหรือไม่ ☐ ไม่พบ ☐ พบ ☐ ไม่ทราบ
2.มีหมัดตามตัวหรือไม่ ☐ ไม่พบ ☐ พบ ☐ ไม่พบตัวแต่พบขี้หมัด ☐ ไม่ทราบ
3.สัตว์มีโอกาสถูกยุงกัดหรือไม่ ☐ ไม่มี ☐ มี ☐ ไม่ทราบ
4.สัตว์เคยมีประวัติเดินทางไปต่างประเทศหรือไม่(เมื่อไหร่) ☐ ไม่เคย☐ เคย.....
5.สัตว์เคยท้องเสียหรือไม่(เมื่อไหร่) ☐ ไม่เคย☐ เคย.....
6.เคยพบปล้องของพยาธิในอุจจาระหรือไม่ ☐ ไม่พบ ☐ พบ ☐ ไม่ทราบ
7.สัตว์เคยอาเจียนหรือไม่(เมื่อไหร่) ☐ ไม่เคย☐ เคย.....
8.เคยมีพฤติกรรมไล่จับสัตว์อื่นหรือไม่ ☐ ไม่เคย☐ เคย
ถ้าเคย ☐ หนู ☐ สัตว์เลี้อยคลาน ชนิด ..... ☐ ปลา ☐ กบ ☐ แมลง ชนิด ..... ☐ อื่นๆ.....

9.สัตว์เคยดื่มน้ำในแหล่งน้ำตามธรรมชาติ ☐ ไม่เคย☐ เคย

Appendix 3

<u>แบบสอบถามโครงการศึกษาความชุกของปรสิตในทางเดินอาหารในแมวในเขตกรุงเทพมหานคร</u>

ID.....

วันที่.....

<u>ข้อมูลพื้นฐาน</u>

ชื่อสัตว์..... พันธุ์ ☐DSH ☐อื่นๆ..... อายุ.....ปี.....เดือน เพศ ☐ผู้ยังไม่ทำหมัน ☐เมียยังไม่ทำหมัน

ชื่อ-นามสกุลเจ้าของสัตว์..... ที่อยู่.....

เบอร์โทรศัพท์ติดต่อ (บ้าน)..... มือถือ.....

จำนวนสัตว์เลี้ยงที่นำมารับบริการ ☐สุนัข.....ตัว ☐แมว.....ตัว ☐อื่นๆ.....

จำนวนสัตว์ทั้งหมดที่เลี้ยง ☐สุนัข.....ตัว ☐แมว.....ตัว ☐อื่นๆ.....

<u>ข้อมูลด้านการเลี้ยง</u>

อาหารที่ให้สัตว์เลี้ยง มีเนื้อสัตว์ดิบเป็นส่วนผสมหรือไม่ ☐ใช่ ☐ไม่ ☐ไม่ทราบ

พื้นที่การเลี้ยง ☐ภายในตัวอาคารเท่านั้น ☐สามารถออกมานอกอาคารได้

มีกระบะทรายสำหรับแมวหรือไม่ ☐มี ในอาคาร / นอกอาคาร ☐ไม่มี

ทรายในกระบะทรายสำหรับแมว ☐สำเร็จรูป ☐ทรายธรรมดา โปรดระบุที่มา.....

ความถี่ในการเปลี่ยนกระบะทราย ☐ทุกวัน ☐มากกว่า 1 วัน

มีหมัดบนตัวสัตว์ใน 1 เดือนที่ผ่านมานี้หรือไม่ ☐มี ☐ไม่มี

มีมาตรการการป้องกันหมัดหรือไม่ ☐มี อย่างไร ยากิน / ยาหยด / ปลอกคอ ☐ไม่มี

มีการถ่ายพยาธิทุก 1 เดือนหรือไม่ ☐มี ☐ไม่มี ทุก.....เดือน/ปี

เคยพบพยาธิออกมากับอุจจาระหรืออาเจียนหรือไม่ ☐มี อุจจาระ / อาเจียน ☐ไม่มี

<u>ข้อมูลพฤติกรรมเสี่ยงต่อการติดโรคปรสิตในทางเดินอาหาร</u>

มีพฤติกรรม ล่า / เล่น / กิน **นก/ไก่** ☐ใช่ ☐ไม่ ☐ไม่ทราบ

มีพฤติกรรม ล่า / เล่น / กิน **หนู** ☐ใช่ ☐ไม่ ☐ไม่ทราบ

มีพฤติกรรม ล่า / เล่น / กิน **กบ** ☐ใช่ ☐ไม่ ☐ไม่ทราบ

มีพฤติกรรม ล่า / เล่น / กิน **ปลา** ☐ใช่ ☐ไม่ ☐ไม่ทราบ

มีพฤติกรรม ล่า / เล่น / กิน **จิ้งจก/จิ้งเหลน** ☐ใช่ ☐ไม่ ☐ไม่ทราบ

มีพฤติกรรม ล่า / เล่น / กิน **แมลงปีกแข็ง** ☐ใช่ ☐ไม่ ☐ไม่ทราบ

<u>ข้อมูลกลุ่มเสี่ยงที่มีโอกาสติดเชื้อจากสัตว์เลี้ยง</u>

มีกลุ่มเสี่ยงฯดังต่อไปนี้ในบ้านหรือไม่

☐เด็กอายุน้อยกว่าหรือเท่ากับ 5 ปี ☐ผู้หญิงท้อง ☐ผู้ป่วยติดเชื้อ HIV

☐ผู้ป่วยที่ได้รับยารักษาโรคมะเร็ง ☐ผู้ป่วยที่ได้รับการปลูกถ่ายอวัยวะ

<u>ข้อมูลเกี่ยวกับการตระหนักถึงความสำคัญของโรคติดเชื้อปรสิตในทางเดินอาหารและการป้องกัน</u>

ท่านทราบหรือไม่ว่าแมวของท่านมีโอกาสติดเชื้อปรสิตในทางเดินอาหารได้ ☐ทราบ ☐ไม่ทราบ

ท่านคิดว่าการติดเชื้อปรสิตในทางเดินอาหารในแมวของท่านมีความสำคัญต่อสุขภาพของแมวมากน้อยเพียงใด

☐มากที่สุด ☐มาก ☐ปานกลาง ☐น้อย ☐น้อยที่สุด

ท่านคิดว่าการตรวจหาเชื้อปรสิตในทางเดินอาหารในแมวของท่านมีความสำคัญมากน้อยเพียงใด

☐มากที่สุด ☐มาก ☐ปานกลาง ☐น้อย ☐น้อยที่สุด

ท่านคิดว่าการป้องกันการติดเชื้อปรสิตในทางเดินอาหารในแมวของท่านมีความสำคัญมากน้อยเพียงใด

☐มากที่สุด ☐มาก ☐ปานกลาง ☐น้อย ☐น้อยที่สุด

ท่านทราบวิธีการป้องกันการติดเชื้อปรสิตในทางเดินอาหารในแมววิธีใดบ้าง (ตอบได้มากกว่า 1 ข้อ)
☐ยาหยดหลัง ☐ยากิน ☐ปลอกคอ
ท่านทราบหรือไม่ว่าปรสิตในทางเดินอาหารบางชนิดในแมวสามารถติดต่อจากสัตว์สู่คนได้ ☐ทราบ ☐ไม่ทราบ
ท่านคิดว่าการติดเชื้อปรสิตในทางเดินอาหารในแมวของท่านมีความสำคัญในแง่ของการนำโรคจากสัตว์สู่คนมากน้อยเพียงใด
☐มากที่สุด ☐มาก ☐ปานกลาง ☐น้อย ☐น้อยที่สุด
ลงชื่อ.....ผู้สัมภาษณ์

Appendix 4

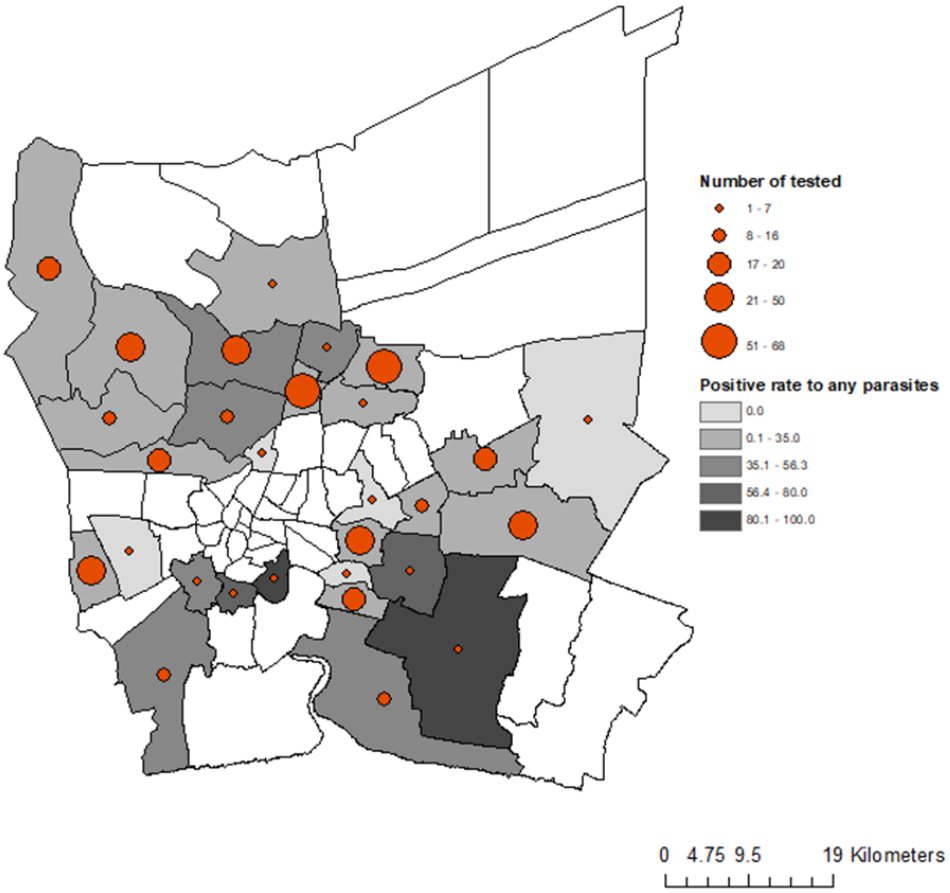
Spatial distribution of enteric parasites infections in Bangkok and vicinities, 2014-2015 (n=509)

Regions/Districts	%ANY (n)	%ANC (n)	%TOX (n)	%PF (n)	%CYS (n)	%TT (n)	%SPI (n)	%DC (n)	%OPI (n)
Total (n=509)	32.0 (163)	21.6 (110)	6.9 (35)	3.7 (19)	3.5 (18)	2.9 (15)	1.6 (8)	0.4 (2)	0.2 (1)
Bangkok (n=339)	30.4 (103)	20.9 (71)	7.7 (26)	2.9 (10)	3.5 (12)	2.1 (7)	1.5 (5)	0.3 (1)	0.3 (1)
Northern Bangkok (n=138)	28.3 (39)	21.7 (30)	5.1 (7)	0.7 (1)	2.2 (3)	2.2 (3)	0.0 (0)	0.0 (0)	0.0 (0)
Eastern Bangkok (n=73)	30.1 (22)	21.9 (16)	5.5 (4)	0.0 (0)	6.8 (5)	1.4 (1)	1.4 (1)	0.0 (0)	0.0 (0)
Southern Bangkok (n=60)	30.0 (18)	18.3 (11)	8.3 (5)	5.0 (3)	5.0 (3)	0.0 (0)	3.3 (2)	0.0 (0)	0.0 (0)
Northern Thonburi (n=7)	42.9 (3)	28.6 (2)	0.0 (0)	28.6 (2)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Southern Thonburi (n=61)	34.4 (21)	19.7 (12)	16.4 (10)	6.6 (4)	1.6 (1)	4.9 (3)	3.3 (2)	1.6 (1)	1.6 (1)
Nonthaburi (n=154)	33.8 (52)	21.4 (33)	3.9 (6)	3.2 (5)	3.9 (6)	5.2 (8)	1.9 (3)	0.6 (1)	0.0 (0)
Mueang Nonthaburi (n=16)	56.3 (9)	18.8(3)	25.0(4)	6.3 (1)	0.0 (0)	6.3 (1)	12.5 (2)	0.0 (0)	0.0 (0)
Bang Kruai (n=20)	25.0 (5)	15.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)	10.0 (2)	0.0 (0)	0.0 (0)	0.0 (0)
Bang Yai (n=14)	28.6 (4)	14.3 (2)	0.0 (0)	0.0 (0)	0.0 (0)	7.1 (1)	0.0 (0)	7.1 (1)	0.0 (0)
Bang Bua Thong (n=50)	26.0 (13)	20.0(10)	2.0 (1)	2.0 (1)	4.0 (2)	2.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)
Sai Noi (n=20)	30.0 (6)	15.0 (3)	0.0 (0)	10.0 (2)	15.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Pak Kret (n=34)	44.1 (15)	35.3(12)	2.9 (1)	2.9 (1)	2.9 (1)	8.8 (3)	2.9 (1)	0.0 (0)	0.0 (0)
Pathum Thani (n=3)	33.3 (1)	33.3 (1)	0.0(0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Mueang Pathum Thani (n=3)	33.3 (1)	33.3 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Samut Prakan (n=13)	53.8 (7)	38.5 (5)	23.1 (3)	30.8 (4)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Mueang Samut Prakan (n=12)	50.0 (6)	33.3 (4)	15.4 (2)	25.0 (3)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Bang Phli (n=1)	100.0 (1)	100.0(1)	100.0 (1)	100.0 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)

ANY; any parasites infection, ANC; *Ancylostoma* spp.., TOX; *Toxocara* spp, PF; *Platynosomum fastosum*, CYS; *Cystoisospora* spp., TT; *Taenia taeniaeformis*, SPI; *Spirometra* spp., DC; *Dipylidium caninum*, OPI; *Opisthorchis* like

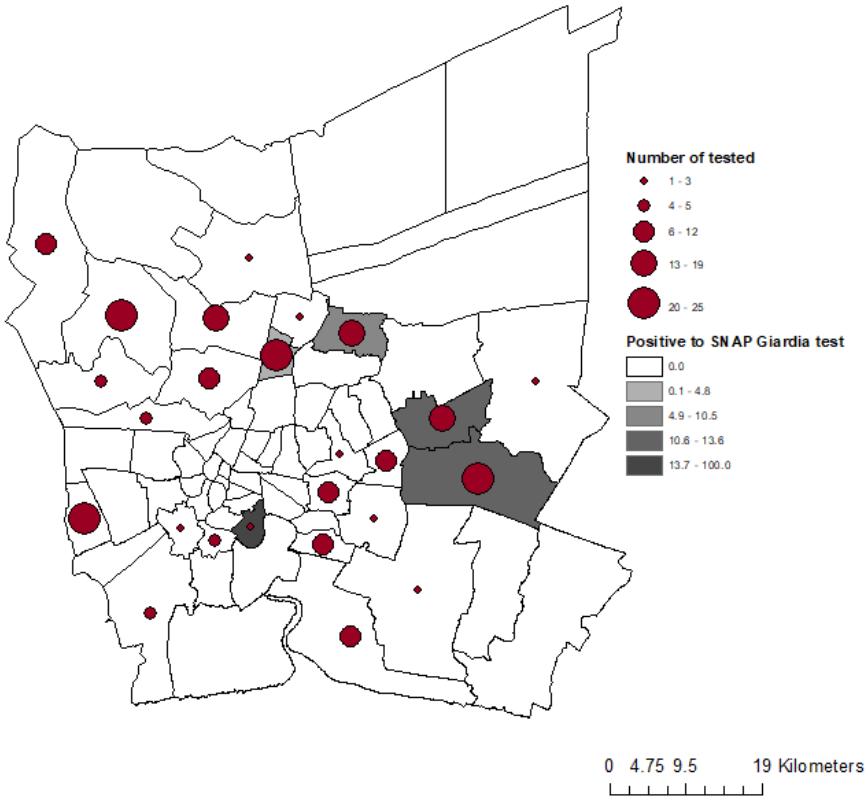
Appendix 5

Spatial distribution of any endoparasites infection in cats in Bangkok and vicinities 2014-2015



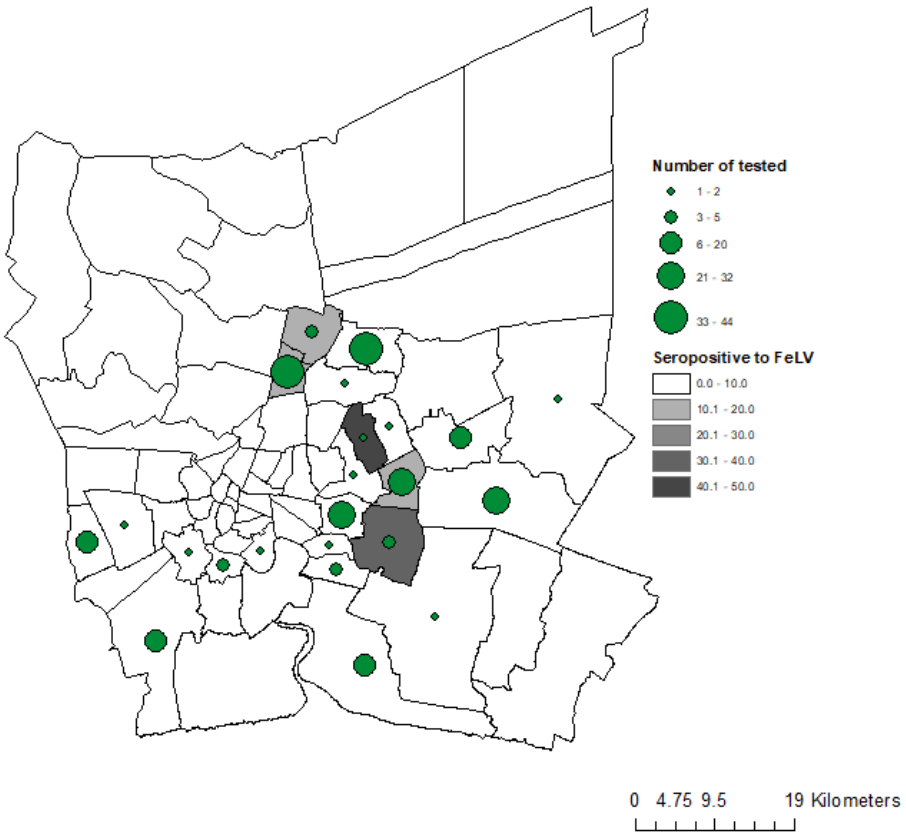
Appendix 6

Spatial distribution of positive for SNAP® *Giardia* in cats in Bangkok and vicinities 2014-2015



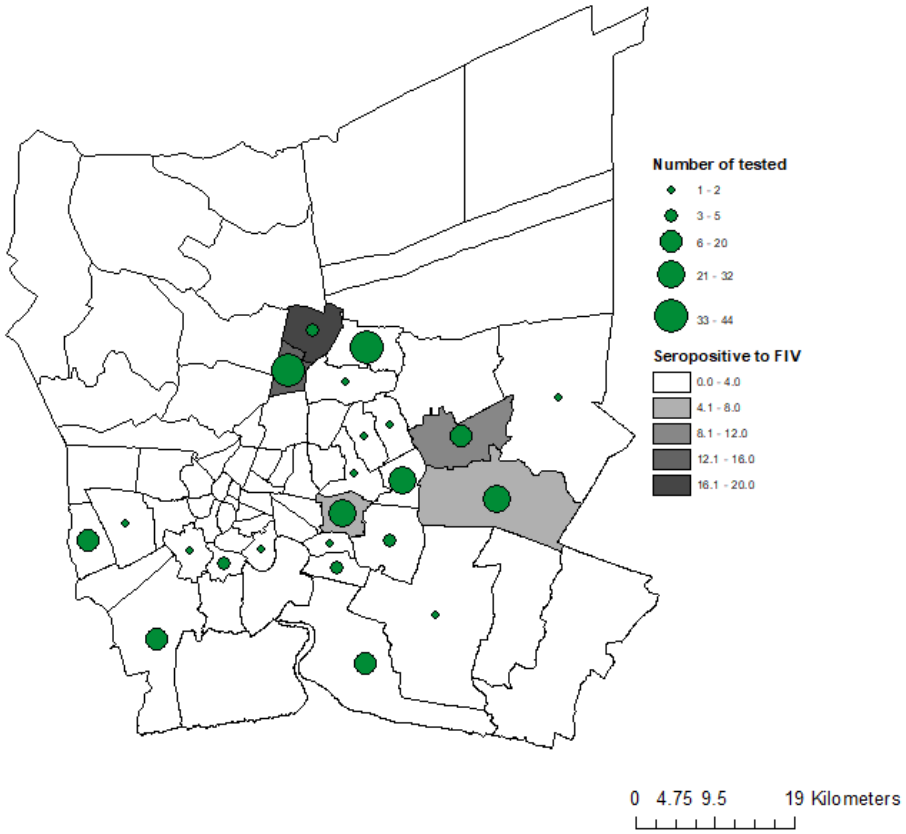
Appendix 7

Spatial distribution of FeLV infection using SNAP® Feline Triple in cats in Bangkok and vicinities 2014-2015



Appendix 8

Spatial distribution of FIV infection using SNAP® Feline Triple in cats in Bangkok and vicinities 2014-2015



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VITA

NAME	Wanarit Jitsamai
DATE OF BIRTH	4 December 1989
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	2008-2013 Doctor of Veterinary Medicine (DVM), Faculty of Veterinary Science, Chulalongkorn University, Thailand 2013-Present Ph.D. candidate in Pathobiology program, Faculty of Veterinary Science, Chulalongkorn University, Thailand
HOME ADDRESS	109/67 Sukhumvit road soi 48, Phra Khanong sub-district, Khlong Toei district, 10110, Bangkok, Thailand