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One Health Approach to Control Zoonotic Disease and Improve Quality of Life

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The Prevalence and Diversity of Hookworm Infections and Strongyloidiasis in Cats and Humans in a Rural Thailand Villages

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Abstract

In southtern Thailand, hookworm infections and strongyloidiasis are prevalent in humans and particularly in rural areas. Yet, information on potentially zoonotic parasites in animal reservoir hosts is lacking. This research would to assess risk potential zoonotic disease of Strongyloidiasis and Hookworm Infection from cats to human In Rural Villages Southtern Thailand. In 2014, fecal samples from 96 cats and 1050 humans, 96 cats were collected from households in Moklalan and Photong villages, Thasala district, Nakhon Si Thammarat province, Thailand. Fecal samples were examined microscopically using modified formal-ether concentration technique and Koga Agar plate culture. PCR and DNA sequencing were used to confirm genomes and species of hookworm. Result of study showed hookworm infections and strongyloidiasis found in cats including hookworms (46%), and *Strongyloides stercoralis* (1,7%). In humans, hookworm (52%), *Strongyloides strercoralis* (13%), *T. trichiura* (14%) and *Ascaris* (1%). Identify species of hookworm is *N. americanus*, but cats usually could be infected by *Ancylostoma caninum* and this study given statement that cats was not equal with human hookworm infections which have not zoonotic potential also *Strongyloides stercoralis*, *Ascaris lumbricoides*. and *T. trichiura* because the cats behavior on defecation made larvae of soil transmitted helminth not completed to infective filariae form larvae especially hookworm and *strongyloidisis strercoralis*. Further environmental epidemiology studies of hookworm infections and strongyloidiasis are important for determination analyses of zoonotic diseases especially in community.

Keywords: Cats, Humans, Hookworm infection and Strongyloidiasis.

1. Introduction

Worldwide, there is a significant variation in the prevalence of gastrointestinal zoonotic helminths in dogs and cats [1,2] Intestinal parasitic infections (IPIs) are the most common infections among humans and domestic animals such as dogs, cats and pigs, particularly in the rural areas of Southeast Asia. Infection of zoonotic helminths has previously been researched in Thailand. In the central area, a high prevalence of hookworm *Ancylostoma ceylanicum* was reported among dogs in temple communities in Bangkok [3]. Chronic infections with one or several of the most common soil-transmitted helminths (STHs), *Ascaris lumbricoides, Trichuris trichiura* and hookworms, might account for a global burden of 39 million disability-adjusted life years lost annually [4]. Another STH, *Strongyloides stercoralis*, is often neglected in helminth surveys [5,6], yet previous studies show high *S. stercoralis* infection rates in Cambodia [7]. School-aged children in the developing world are at highest risk of morbidity due to STHs and intestinal protozoan infections [8]. Many of the IPIs in animals, especially those with the larval stages of hookworms, *Gnathostoma spp*. and *Toxocara spp*., may result in zoonotic diseases such as eosinophilic enteritis [9], cutaneous larval migrans, and toxocariasis.

However, mass treatment only focuses on three major STHs (*Ascaris*/hookworm/*Trichuris*). Other nematodes like *S. stercoralis*, trematodes and protozoan infections are not addressed. In rural

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Southeast Asia, little is known about the zoonotic potential of IPIs in humans and animals. Therefore of domestic animals, such as cats, dogs and pigs, as contributors to human IPI and as reservoir hosts for zoonotic parasites remains unexplored and/or the data are inaccessible.

Although surveys of zoonotic gastrointestinal helminths in dogs and cats had been done in Thailand, most of the studies have focused on the Central or Northeastern region [10, 11, 12, 3]. This was the first study to investigate prevalence of zoonotic helminth infection in humans and cats in Southern area of Thailand introductions, or conclusions.

2. Methods

2.1. Ethical considerations

The study protocol was approved by the Ethics Committee of Walailak University, All participants and relevant parties were informed of the purpose of the study. Written informed consent was obtained from all individuals prior to enrolment. All infections diagnosed in humans and animals were treated at the end of the study according to the Thai treatment guidelines.

2.2. Study design and area

The study was carried out in May 2014 in two villages such as Mokhalan and Pothong villages, Thasala district, Nakhon Si Thammarat province, Southern Thailand). The climate is tropical, with warm and hot temperatures all year round and alternating dry and wet seasons. Households from Mokholan and Phothong villages were randomly selected from lists provided by the Ministry of Health. All household members (N2 years) and animals (cats) were assessed for IPIs using a single diagnostic test approach on one stool sample for each human and one sample for each animal. Only animals owned by the household were included in the survey. Risk factors for infection of humans and animals were assessed based on information collected through questionnaire interviews and observations.

2.3. Field procedures and sample collection

On the day of the first visit, informed consent was obtained from all household members and interviews were conducted with enrolled participants. Interviews with young children were conducted with the help of a parent or legal guardian. All enrolled participants received a prelabelled stool container. Participants were asked to fill the container with faeces passed the following morning. Upon collecting the first sample, a second stool container was given to participants for filling. The collected stool samples were transported within two hours following defecation to a laboratory in Medical Tehnology laboratorium Walailak University. Stool samples from each human and cat present at the time of the visit and belonging to the household were obtained. For each animal, approximately five grams of faeces were collected from around houses, placed into a sterile plastic faecal container and chilled immediately in a box containing ice. For each human, one stool sample given on consecutive day was analyzed and for each animal, one sample was analyzed.

2.4. Laboratory procedures

For each human stool sample, the following tests were performed: Kato Katz [13], Koga Agar culture [14], formalin-ether concentration technique (FECT) [15] analysis. As they arrived in the laboratory, human samples were processed as follows:

First, duplicate Kato Katz smears were prepared. Stool was filtered using a nylon mesh and then placed on the standard Kato Katz template, leaving 41.7 mg of stool for examination on a microscopic slide. Examination was performed at 100x magnification [13] for hookworm and *S. stercoralis*.

Second, a Koga Agar test was prepared by placing a piece of stool (3–5 g) on a freshly produced Agar plate. The plates were then incubated for 48 hours at 28 °C. Larvae were washed from the plate into a tube, the liquid was centrifuged and the entire sediment was read at 40x magnification [14] for hookworm and *S. stercoralis* larvae. Additionally, in this research only for humans samples were fixed in 2.5% potassium dichromate for subsequent PCR screening for hookworm [16].

3. Results and Discussion

Humans and Cats samples In this study, fecal samples from 96 cats and 1050 humans, 96 cats were collected from households in Moklalan and Photong villages, Thasala district, Nakhon Si Thammarat province, Thailand. All this area closed by rubber and palm plantations and have distance 20 km away from sea area.

Prevalence in Animals Result of study showed hookworm infections and strongyloidiasis found in cats including hookworms (46%), and *S.stercoralis* s (1,7%).

Table 1. STHs Infection in Animals

Parasite	Cats
Hookworm	48% (46/96)
Strongyloides stercoralis	1.7% (1/96)

Dogs and cats are important reservoir hosts of various zoonotic helminths [17, 18, 2], many of which cause serious public health problems. Here, we reported the prevalence of zoonotic intestinal helminths in lower Northern Thailand as 40.1 % (79/197) in dogs and 33.9 % (61/180) in cats, respectively. Zoonotic helminths found
included hookworms, *Spirometra* spp., *Toxocara*, *O. viverrini*, *Taenia* spp. *Strongyloides* and *Trichuris* [19,20].

Zoonotic hookworm, *A. caninum*, was found to have low infection rates in both dogs and cats. Similar to other areas, prevalence of *A. caninum* was lower than that of *A. ceylanicum* [33, 34]. Although its infection rate was low, this hookworm can result in eosinophilic enteritis and chronic abdominal pain in human [8,16]. Other zoonotic hookworm, such as *A. braziliensis*, was not found in this area.

Among zoonotic helminth infections in dogs in the lower Northern area of Thailand, hookworms were the most prevalent helminth, and *Spirometra* was the second most prevalent. Our results confirmed that hookworm infection in dogs is common in Thailand [12]. The high prevalence of hookworm infections in dogs can contribute to the occurrence of zoonotic ancylostomiasis in human [21].

Zoonotic helminth infections in cats were different from dogs. *Spirometra* was the most prevalent, while hookworms were the second most prevalent helminth. High rates of *Spirometra* infection might be a reflection of the fact that most cats roam freely and had access to small prey as a food source. High infection rates of *Spirometra* spp. in cats might indicate a high infection rate of plocercoid and plerocercoid in intermediate hosts in the area. The infection of *Spirometra* spp. in cats and dogs can lead to a high risk of sparganosis in humans who have the habit of eating undercooked meat [22, 23]. However, human sparganosis in Thailand is rare. In the period 1943-2010, only 53 cases had been reported [22, 23].

Dogs are associated with more than 60 zoonotic parasites worldwide, many of which pose serious public health concerns [24]. Compared with some other studies in South Asian countries, the overall prevalence of IPIs in dogs in Cambodia was higher (81.9%) than previously reported for dogs in rural India, for example [25].

This research in contrast with Combodian research that dogs in rural Cambodian villages such as Dong village are largely kept as guard dogs and allowed to roam freely, especially during the day. The dogs are also allowed inside the house and around rice and vegetable fields and ponds. At night-time, the dogs then often stay in or around the house. Dogs, therefore, pose a serious zoonotic risk as they have the potential to transmit zoonotic parasites through their close association with household members as well as through heavy contamination of the environment, including soil, fresh produce and waterways, with parasite eggs and oocysts, in our observation sew behavior of cats almost all day and night stayed around houses and rare contact with ponds and did not stay around rice and vegetable fields.

Prevalence Soil Transmitted Helminths/STHs infection in Humans such as, hookworm (52%), *S.stercoralis* (13%), *T. trichiura* (14%) and *Ascaris* (7%).

Table 2. Prevalence STHs Infection in Human

Parasite	Humans
Hookworm	52% (546/1050)
S.stercoralis	13% (137/1050)
T. Trichiura	14% (147/1050)
Ascaris	7% (73/1050)

The present study showed similar patterns of IPIs in humans compared to previous surveys conducted in Cambodia [4,33,34]. The major IPIs found in humans were hookworms (63.3%), Entamoeba spp. (27.1%), *S. stercoralis* (24.3%), *G. duodenalis* (22.0%) and *Blastocystis* (18.4%) . In total, 14 different parasite species were diagnosed, including eight helminthic and six protozoan parasites. Of the 218 participants, 27 (12.8%) were negative in all examinations. More than a quarter of the human participants (64, 29.4%) were infected with one parasite and a third (72, 33.0%) with two or more parasites. Three (1.4%) and one (0.5%) participant(s) harboured five and six parasites, respectively, the prevalences of parasites (those with the highest infection rates) are given for the different age-groups. For hookworm, the prevalence increases from less than 50.0% in children up to the age of ten to more than 60.0% in adolescents and then remains above 60.0% in all subsequent age-groups. For *S. stercoralis*, the prevalence also increases over age, reaching its peak in age-groups 30 years and older. Fig. 3 shows that the average number of helminthic co-infections increases over age, whereas the average number of protozoan co-infections is highest in children and lowest in adults older than 51 years. However, in this study, no cases of human *Ascaris spp*. infection were detected by microscopy. This coincides with the findings of Park and colleagues [26]. We demonstrated that in all age-groups, the average number of co-infections is about the same, yet helminthic co-infections accumulated over time, with a peak in 30–50 year old individuals. The trend for protozoan co-infections is reversed, with the highest number of protozoan co-infections occurring in children. This pattern might reflect higher exposure of children. Alternatively, it could be because of higher infection intensities rather than prevalence in children, as microscopy can miss low-intensity protozoan infections [27], although this applies also for helminthic infections.

DNA Analysis, Identify species of hookworm is *N. americanus*, but cats usually could be infectived by *Ancylostoma caninum*, condition of data this research not similar with research in Northern Thailand that it has significant zoonotic hookworms include A. ceylanicum, A. braziliensis and *A. caninum* [28, 21, 29]. Molecular analysis revealed that the most prevalent hookworm (over 80%) found in dogs and cats in the lower Northern area was *A. ceylanicum. A. ceylanicum* is highly prevalent in many areas in Asian countries [29, 33, 34] and is known to produce potent infections in humans. *A. ceylanicum* is the second most common hookworm infection in humans that can lead to anemia [28, 21].

PCR and sequencing were used for detection and identification of parasites in various specimens with high sensitivity and specificity [3, 30]. In our survey, molecular analysis was applied for two significant helminths infection, hookworms and *O. viverrini*. Morphological identification of hookworm larvae or eggs to species is difficult, and molecular identification provides great results in this regard [20].

This research has deference with research in Cambodia which it showed that in humans about half of the infections (51.6%) were *Ancylostoma ceylanicum* and the remaining *Necator americanus* infections. In dogs over 90% were *A. ceylanicum* indicating that most probably dogs are the source of infection. We hypothesize that regular deworming in communities lead to a replacement of *N. americanus* by *A. ceylanicum*. Parallel deworming of the dog population is likely to reduce the incidence in humans [31]

Zoonotic Risk factors, in this research could see via behavior of defecation cats and environmental factors also personal hygiene and sanitation have contribution became spread STHs infected from animal to human, defecation of cats observation had not potential risk infection from cats to human because cats usually closed with soil after defecated and cat anly defecated round houses and the fecal dry by solar contact then parasite was killed, deferent with studied in Nortern Thailand that Zoonotic hookworm, *A. caninum*, was found to have low infection rates in both dogs and cats. Similar to other areas, prevalence of *A. caninum* was lower than that of *A. ceylanicum* [3, 20]. Although its infection rate was low, this hookworm can result in eosinophilic enteritis and chronic abdominal pain in human [32, 33, 34, 35].

Environmental factors have potential zoonotic determined of hookworm infection and strongylidiasis where this area researched location have poor sanitation, without wastewater drainage that made wet surrounding houses and sandy soil round houses could make easy to spread hookworm and *S.stercoralis* by directed penetration from cats to human, defecated cats could not spread hookworm infection and strongyloidiasis because cats behavior on defecation made larvae of soil transmitted helminth not completed to infective filariae form larvae especially hookworm and *S.stercoralis*

4. Conclusions

This study given statement that cats was not equal with human hookworm infections which have not zoonotic potential also *Strongyloides stercoralis, Ascaris lumbricoide*s. and *T. trichiura* because cats behavior on defecation made larvae of soil transmitted helminth not completed to infective filariae form larvae especially hookworm and strongyloidisis strercoralis. Further environmental epidemiology studies of hookworm infections and strongyloidiasis are important for determined analysis zoonotic diseases especially in community.

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