RESEARCH ARTICLE
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Environmental soil contamination with zoonotic soil-transmitted helminths in the temple grounds of Chachoengsao Province, Thailand

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Received: 30-11-2022, **Accepted:** 07-02-2023, **Published online:** 17-04-2023

doi: www.doi.org/10.14202/IJOH.2023.15-20 **How to cite this article:** Dokmaikaw A and Suntaravitun P (2023) Environmental soil contamination with zoonotic soil-transmitted helminths in the temple grounds of Chachoengsao Province, Thailand, *Int. J. One Health*, 9(1): 15–20.

Abstract

Background and Aim: Soil-transmitted helminth infections are a serious public health problem. The soil is an important reservoir for several helminth species. This study aimed to estimate the prevalence of soil contamination with zoonotic soil-transmitted helminths.

Materials and Methods: Soil samples were collected on the grounds of 19 temples in Ratchasan and Bang Khla districts, Chachoengsao Province, Thailand, between June 2021 and February 2022. A total of 190 soil samples were collected from ten different areas on the grounds of each temple, of which 31 (16.3%) were contaminated. The samples were analyzed by a centrifugal flotation technique using a saturated sodium nitrate solution.

Results: Six genera of helminths were recovered from the soil samples, of which five genera were identified as helminth eggs and two genera were identified as helminth larvae. The prevalence of soil contamination with helminth eggs was as follows: *Toxocara* spp. 15.8% (30/190), hookworm/strongylid 12.1% (23/190), *Trichuris* spp. 6.8% (13/190), *Taenia* spp. 2.1% (4/190), *Ascaris* spp. 1.6% (3/190), and unidentified helminth eggs 5.8% (11/190). Moreover, nematode larvae consisted of 8.4% (16/190) hookworm/strongylid, 3.7% (7/190) of *Strongyloides* spp., and 4.2% (8/190) unidentified nematode larvae. The rates of soil-transmitted helminth contamination were not significantly different between Ratchasan and Bang Khla districts (p = 0.878).

Conclusion: Soil-transmitted helminth contamination was found in the soil environment of the community temples with a high probability of spreading intestinal helminthiasis to the local residents. Therefore, public awareness campaigns should be conducted targeting people in the community to increase their knowledge and understanding about animal healthcare to prevent and control the spread of soil-transmitted helminthiasis.

Keywords: soil contamination, soil-transmitted helminth, temple, Thailand, zoonotic helminth.

Introduction

The spread of soil-transmitted helminthiasis is a serious global health problem. The soil is an important reservoir for several species of helminths. Helminthiasis can be transmitted to animals and humans by ingesting food contaminated with helminth eggs or larvae and cutaneous penetration with the larval stages of helminths. Hookworm, *Toxocara* spp., *Trichuris* spp., *Ascaris* spp., *Strongyloides* spp., and *Taenia* spp. are the common helminth species found in dogs and cats and detected in the soil [1–3]. Cats and dogs are effective reservoir hosts for several zoonotic soil-transmitted helminths and play crucial roles in disease transmission. Defecation and the scavenging behavior of stray cats and dogs within their roaming ranges in a human environment contribute to soil pollution. Fecal materials promote the spread of soil-transmitted helminth infections through human contact with soil contaminated with either the eggs or larval stages of helminths. Several countries have also reported helminth contamination in soil samples collected from various locations, including school compounds, public parks, dumpsite areas, temple grounds, households, and open markets in urban and rural areas [2–13].

Thailand has a large population of semi-domesticated and stray dogs that frequent temple grounds. Therefore, helminth contamination is often present in the soil environment of community temples with a high risk of exposure to several important communicable pathogens that could transmit diseases from animals to humans. Previous studies have reported a high prevalence of 10.4%–18.3% of zoonotic soil-transmitted helminths in Thailand [3, 5–7]. However, no studies have been monitoring environmental contamination by soil-transmitted helminths in Chachoengsao Province.

Therefore, this study aimed to estimate the prevalence of soil contamination with zoonotic soil-transmitted helminths. Soil samples were collected from temple grounds in Ratchasan and Bang Khla districts, Chachoengsao Province, Thailand, and parasitic ova were recovered using the centrifugal flotation technique.

Materials and Methods

Ethical approval

Ethical approval was not required for this study.

Study period and location

The study was conducted from June 2021 to February 2022. Soil samples were collected from 19 temples located in Ratchasan and Bang Khla districts. The study area was 100 km east of Bangkok with a total area of 362.8 km^2 according to the geographic coordinates 13°62′10.8″N to 13°88′12.0″N and 101°13′84.9″E to 101°33′23.6″E (Figure-1). The average annual temperature ranges from 22.8°C to 33.0°C, with an annual rainfall of 1294.0 mm.

Sample collection

A total of 190 soil samples were collected from 10 different locations in each temple by focusing on where the semi-domesticated dogs and cats commonly resided and the walking areas. Samples weighing 200 g were collected by scraping off the surface soil with a metal spoon to a depth of 2 cm. The samples were stored in plastic bags and transported to the laboratory of the Department of Public Health, Faculty of Science and Technology, Rajabhat Rajanagarindra University, Chachoengsao Province, for analysis.

Detection of eggs

The soil samples were examined for the presence of parasites by a centrifugal flotation technique using a saturated sodium nitrate solution [14]. Briefly, each sample was passed through a 150 μm mesh sieve to remove any sticks and stones. Then, 20 g of soil was placed in an Erlenmeyer flask containing 50 mL of 5% sodium hydroxide (NaOH) solution, mixed well, and left for 1 h to separate helminth eggs from soil particles. The samples were then shaken for 20 min, and the suspension was transferred to a centrifuge tube and centrifuged at 327× *g* for 3 min. The resulting supernatant was removed and replaced with water to wash the sample before adding a saturated sodium nitrate ($NaNO_3$) solution with a specific gravity of 1.30 followed by centrifugation at 327× *g* for 3 min. Sodium nitrate solution was added to the brim of the centrifuge tube using a pipette until an upper meniscus was formed. A coverslip was carefully placed on the meniscus to collect the topmost portion. After 10 min, the coverslip was removed and placed on a clean glass slide for examination under a light microscope for the presence of soil-transmitted helminths.

Statistical analysis

Data analysis was conducted using the Statistical Package for the Social Sciences for Windows, version 23 (IBM Corp. NY, USA). The prevalence of soil contamination with soil-transmitted helminths was determined as frequencies and percentages. Fisher's exact test was used to compare differences in contamination rates according to the soil-transmitted helminth species and type of contamination (single, double, triple, and quadruple helminths). $p < 0.05$ indicated a statistically significant difference between mean values.

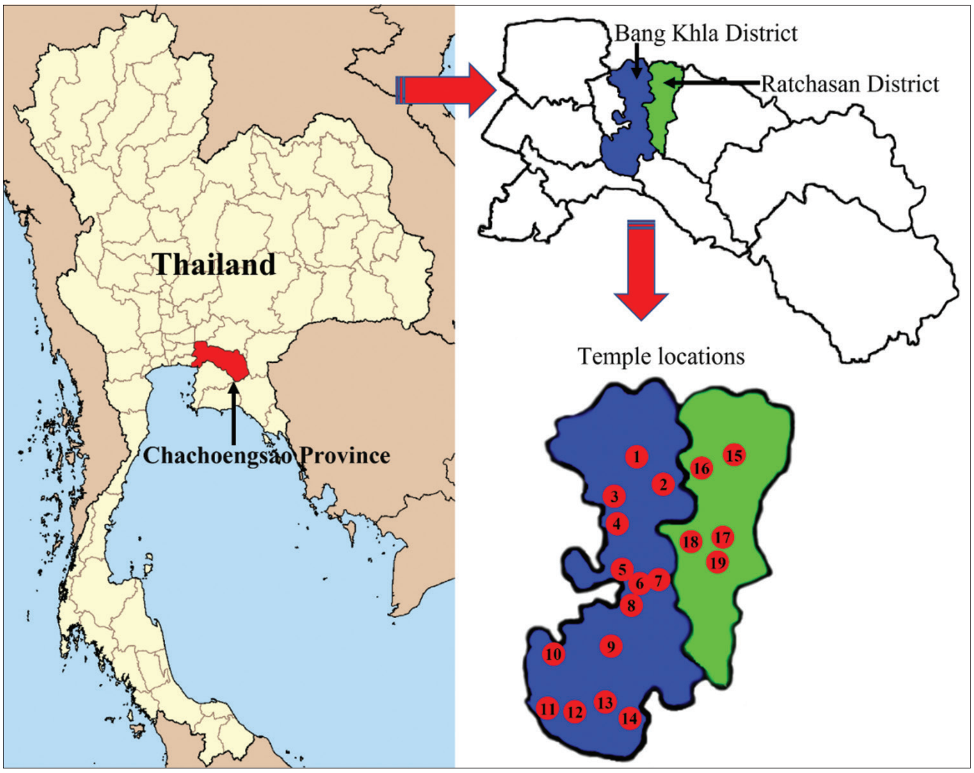

Figure-1: Map of Thailand showing the temple locations in Ratchasan and Bang Khla districts, Chachoengsao Province, Thailand [Source: https://commons.m.wikimedia.org/wiki/Atlas_of_Thailand#].

Results

Of the 190 soil samples collected from temples in Chachoengsao Province, 50 samples were collected from Ratchasan district, and 140 samples were collected from Bang Khla district. The overall soil contamination rate with soil-transmitted helminths was 16.3% (31/190). Six genera of helminths were recovered from the soil samples, of which five genera were identified as helminth eggs, and two genera were identified as helminth larvae. The prevalence of soil contamination with helminth eggs was as follows: *Toxocara* spp. 15.8% (30/190), hookworm/strongylid 12.1% (23/190), *Trichuris* spp. 6.8% (13/190), *Taenia* spp. 2.1% (4/190), *Ascaris* spp. 1.6% (3/190), and unidentified helminth eggs 5.8% (11/190). Regarding contamination with nematode larvae, there were 8.4% (16/190) of hookworm/strongylid, 3.7% (7/190) of *Strongyloides* spp., and 4.2% (8/190) of unidentified nematode larvae. No significant differences were observed in the prevalence rates of soil contamination with the soil-transmitted helminths between Ratchasan and Bang Khla districts (p = 0.878) (Table-1). The proportions of recovered helminths were *Toxocara* spp. 26.1%, hookworm/strongylid eggs 20.0%, hookworm/strongylid larvae 13.9%, *Trichuris* spp. 11.3%, *Strongyloides* spp. 6.1%, *Taenia* spp. 3.5%, and *Ascaris* spp. 2.6%. The proportions of other unidentified helminth eggs and unidentified nematode larval stages were 9.5% and 7.0%, respectively. Of the total 190 soil samples, 7 (3.7%) were contaminated with one genus of helminths, 22 (11.6%) were contaminated with two genera of helminths, 1 (0.5%) was contaminated with three helminth genera, and 1 (0.5%) was contaminated with four helminth genera. No significant differences (p = 0.146) existed between the numbers of helminth genera in the soil samples collected from each district (Table-2).

Discussion

The overall prevalence of soil contamination by the eggs and larvae of soil-transmitted helminths with zoonotic potential in the soil samples collected from the temple grounds of Ratchasan and Bang Khla districts of Chachoengsao Province was 16.3%. This value was slightly higher than previously reported from Bangkok Province in the Central Region of Thailand, where 14.0% of the soil samples collected from temple grounds were contaminated [3]. Our result was also higher than previously reported values from Nigeria (10.4%) [4], but lower than those recorded in Malaysia (23.0%) [2], Ethiopia (66.5%) [8], Brazil (72.5%) [12], Iran (51.2%) [13], the Philippines (41.3%) [15], Iraq (48.0%) [16], and Poland (41.4%) [17]. These differences were due to geographical variations, area of the soil sample, number of stray dogs and cats in the area, removal of feces, urban or rural locations, and methods for detecting soil contamination. Our findings revealed no significant differences in the rates of soil contamination between Ratchasan and Bang Khla districts (p = 0.878), because the soil samples were collected from nearby locations.

Toxocara eggs were the most common soil-transmitted helminths (15.8%) in the soil samples. This result was slightly different from a previous report where contamination was detected in 12.0% of the soil samples collected from schools in Sakon Nakhon Province [6] in the northeastern region and in 18.3% of the soil samples in Nakhon Si Thammarat Province [7] in the southern region. Our results revealed higher contamination rates than those in Iran (13.0%) [18], Sri Lanka (12.0%) [19], Poland (1.6%) [20], and Brazil (0.5%–8.3%) [12, 21], but lower than those reported in Iran (18.0%–47.5%) [9, 22, 23], Malaysia (24%) [2], Poland (20.7%) [17], Russia (23.3%) [24], Brazil (27.3%–38.4%) [25, 26], Greece (17.1%) [27], Peninsula (26.6%) [28], Portugal (63.2%) [29], Serbia (22.0%–26.0%) [30], Turkey (19.4%) [31], and Germany (23.2%) [32]. *Toxocara* spp. was found in greater numbers than other helminths, because the soil was contaminated with fecal material from infected dogs and cats, and *Toxocara* spp. was the most common parasitic nematode causing functional digestive

Table 1: Helminth genera contamination rates in the soil samples from Ratchasan and Bang Khla districts of Chachoengsao Province.

Parasite species	Location		Total (n=190)	p-value
	Ratchasan (n=50)	Bang Khla (n=140)		
	Number of positive (%)	Number of positive (%)	Number of positive (%)	
Helminth eggs				0.878
Ascaris spp.	0 (0.0)	3 (2.1)	3 (1.6)	
Hookworm/strongylid	5 (10.0)	18 (12.9)	23 (12.1)	
Toxocara spp.	9 (18.0)	21 (15.0)	30 (15.8)	
Trichuris spp.	4 (8.0)	9 (6.4)	13 (6.8)	
Taenia spp.	1 (2.0)	3 (2.1)	4 (2.1)	
Unidentified helminth eggs	3 (6.0)	8 (5.7)	11 (5.8)	
Nematode larvae				
Hookworm/strongylid	7 (14.0)	9 (6.4)	16 (8.4)	
Strongyloides spp.	2 (4.0)	5 (3.6)	7 (3.7)	
Unidentified nematode larvae	2 (4.0)	6 (4.3)	8 (4.2)	

Table 2: Soil samples contaminated with single, double, triple, and quadruple helminths in Ratchasan and Bang Khla districts of Chachoengsao Province.

Type of contamination	Location		Total (n=190)	p-value
	Ratchasan (n=50)	Bang Khla (n=140)		
	Number of positive (%)	Number of positive (%)	Number of positive (%)	
Single	2 (4.0)	5 (3.6)	7 (3.7)	0.146
Double	9 (18.0)	13 (9.3)	22 (11.6)	
Triple	1 (2.0)	0 (0.0)	1 (0.5)	
Quadruple	0 (0.0)	1 (0.7)	1 (0.5)	
Total	12 (24.0)	19 (13.6)	31 (16.3)	

disorders in dogs and cats worldwide [33]. Our findings demonstrated that the major intestinal parasite species infecting dogs and cats in the soil samples was *Toxocara* spp., which poses a significant risk of zoonotic transmission to humans. In particular, children are at an increased risk for infection due to their poor hygiene practices and play behaviors. The two primary forms of toxocariasis are visceral larva migrans (VLM) and ocular larva migrans, with VLM primarily being a disease of young children [34]. Therefore, there may be a risk of further infection for children living in areas, where *Toxocara* spp. contamination has been discovered in the environment.

Hookworm/strongylid eggs were the second most common soil-transmitted helminths in the soil samples, which concurred with a previous study conducted in Bangkok Province in Central Thailand [3]. The rate of contamination with hookworm/strongylid larval stage in the soil samples was 8.4%. Other published studies have also reported soil contamination rates at 10.38% in the Upper Northern Region of Thailand, including Lampang Province (18.7%) [5], Chiang Mai Province (13.3%) [5], Phayao Province (7.1%) [5], and Chiang Rai Province (2.0%) [5], revealing wide-ranging variations as demonstrated by microscopic examination. Different species of hookworm/strongylid were not considered in this study. A previous study examined fecal samples from dogs to identify hookworm species and determined *Ancylostoma ceylanicum* (96.6%) [5] as the most common. Hookworm infection is a major public health problem associated with helminthiasis in Thailand. The Ministry of Public Health of Thailand conducted a national study on people during the fiscal year 2019 and found that hookworm (4.4%) [35] had the highest prevalence compared with other species belonging to the soil-borne helminth group.

Trichuris spp. eggs were found in the soil samples at a contamination rate of 6.8%. In contrast, the previous studies demonstrated a wide range of soil contamination rates in other countries (2.1%–13.8%) [17, 20], such as the Philippines (5.3%–21.11%) [15], Argentina (3.8%) [36], Iraq (16.7%) [16], Iran (0.4%) [13], and Romania (8.8%) [11]. *Trichuris* spp. can be found in various herbivorous mammals, carnivorous mammals, pigs, and humans; infections are species-specific. Consequently, the geographic distribution of *Trichuris* spp. varies in each area, which may significantly affect the application of appropriate and successful control methods in various areas. *Taenia* spp. were detected in 2.1% of the soil samples, which was consistent with previous reports from the Philippines (0.6%–2.2%) [15] but lower than the contamination rates in Turkey (6.8%) [31] and Iraq (25.0%) [16]. *Ascaris* spp. was found in 1.6% of the soil samples, which was consistent with reports in Argentina (1.1%) [36] but lower than those reported in Malaysia (4.0%) [2], the Philippines (10.0%–25.5%) [15], Nigeria (8.8%–25.1%) [4, 10], and Iraq (33.3%) [16]. The eggs of *Ascaris suum* and *Ascaris lumbricoides* are similar, but the former is considered as a zoonotic helminth parasite, and the latter is specific to humans. Despite the ongoing debate surrounding the classification of these species as separate species or a single species, the transmission potential of infection to humans is extremely important.

Strongyloides spp. larvae were detected in 3.7% of the soil samples, which is consistent with that in Romania (4.4%) [11] but less than that in Nigeria (1.6%–50.2%) [4, 10]. It is difficult to identify *Strongyloides* species in soil samples by microscopic examination due to the possibility of contamination with feces from various mammals; moreover, they are difficult to distinguish if not found in fecal matter. Therefore, applied molecular diagnostic approaches for the taxonomic identification of potentially zoonotic species should be used for accurate detection. The results from the previous studies are varied due to a variety of factors, including the differences in the geographical location of each community. This study detected unidentified helminth eggs and nematode larvae in soil samples that were possibly contaminated by bird droppings, chickens, and bats living on the temple grounds.

According to our findings, the primary genera in the soil survey were *Toxocara* spp. and hookworm/strongylid. This result concurred with the previous studies reporting that these two types of helminths were often detected in dog fecal samples and soil samples from Thai temples [3, 5, 33, 37]. However, several other potentially zoonotic soil-transmitted helminths were recorded in the soil samples, including *Trichuris* spp., *Strongyloides* spp., *Taenia* spp., and *Ascaris* spp. Although dogs and cats certainly transmit a large portion of the identified parasites, it is necessary to consider that various animals, including birds, mammals, and even humans, can contribute to soil contamination. Therefore, more advanced and specific identification techniques must be applied to ensure that all identified parasitic elements are zoonotic.

Conclusion

Zoonotic soil-borne helminth contamination was detected in the soil environment of community temples, indicating a high probability of spreading intestinal helminthiasis to people living in their vicinity. Surveillance of the occurrence of helminth infections should be conducted in areas where stray or semi-domesticated dogs and cats reside. Public awareness campaigns should also be implemented targeting people in the community to increase their knowledge and understanding about animal healthcare to prevent and control the spread of zoonotic infectious diseases.

Authors' Contributions

AD and PS: Conceptualization and design of the study, performed laboratory experiments, data analysis, and wrote the manuscript. Both authors have read, reviewed, and approved the final manuscript.

Acknowledgments

The authors would like to thank the Institute of Research and Development, Rajabhat Rajanagarindra University, Chachoengsao Province, Thailand, for financial support, and the public health students who helped to collect the soil samples.

Competing Interests

The authors declare that they have no competing interests.

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