Zoonotic Helminth Contamination of the Environment in Rural Villages of Southern Lao PDR

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Abstract

Helminth infections are common among humans and animals in Savannakhet Province, Lao PDR. To assess the level of such environmental contamination, surface soil samples, houseflies, and vegetables, were collected from Ban Lahanam and Ban Thakhamlaine villages, in Songkhone District. Surface soil samples contained one ascarid egg, as well as free-living nematode larvae. The external body surfaces of the houseflies carried adult Haplorchis taichui (otherwise known as minute intestinal fluke), as well as opisthorchid and taeniid eggs, nematode larvae, and mites. Three third-stage angiostrongylid larvae were found on pre-washed vegetables purchased at a local market. Taeniid, oxyurid, trichurid and strongylid helminths eggs were detected on other vegetable samples. Such findings indicate this environment was contaminated with several different helminth species at various stages of development, highlighting the risk of parasitic zoonotic infections for humans and domestic animals. Communities should therefore be offered advice on how to improve standards of sanitation.

Keywords: zoonotic helminth infection, environmental contamination, Lao PDR

Introduction

Inhabitants of rural areas in a number of developing countries continue to display high prevalence rates of helminthic infection. Zoonotic parasitic infections, especially, tend to occur in areas where animals and humans have close contact. In some remote areas of Lao PDR, humans and animals often share the same living space, being kept either under the same roof or within reachable distance. Household environments are easily contaminated with animal dung, making living conditions unsanitary. Pathogens in...
fecal matter also accumulate in soil and can be carried elsewhere by flies, animals and humans. Animal dung is frequently used as a fertilizer for homegrown vegetables, which in turn can result in the contamination of food with pathogenic organisms. These vegetables can later become a source of infection for humans, most commonly with zoonotic diseases. Our previous studies found several helminthes to be prevalent in the village of Ban Lahanam in Savannakhet, including *Opisthorchis viverrini*, *Haplorchis taichui*, hookworm, *Taenia* sp and *Trichostrongylus colubriformis* [1-3]. To evaluate the potential risk of infection, this study sought to detect helminth objects present in the environment by examining surface soil samples, houseflies and vegetables – which are the suspected sources of helminthiases transmission – in the villages of Ban Lahanam and Ban Thakhamlaine in the Songkhone District of Savannakhet, Lao PDR.

**Materials and methods**

**Study sites**

Our previous studies confirmed the southern Laotian province of Savannakhet to be an endemic area for several species of zoonotic helminths, thus making it a suitable site for this study [1-3]. Ban Thakhamlaine and Ban Lahanam, two villages in the Songkhone District, were chosen. Both are adjacent without any clear boundary; located at latitude 16° 16’ 0" N and longitude 105° 16’ 0" E. Household and villager demographics, as well as environmental surroundings, are almost identical (Fig 1). A small morning market located near the health office was used. Three types of samples were collected from the study areas: surface soil, houseflies, and vegetables. They were transported to the laboratory of the Faculty of Tropical Medicine, Mahidol University, in Bangkok, for examination.

![Fig 1 The two study areas.](image)
Soil samples

Surface soil was found to play a crucial role in the infection/reinfection of community members [4,5]. Samples of surface soil were collected from various sites around each house, including the septic tank (n = 11) and the playground or animal-keeping area (n = 15). In the chosen areas, a sample of surface soil, measuring 1 sq ft with no greater depth than 1 cm, was taken and kept in an individually labeled plastic bag. All samples were kept at room temperature and subsequently transferred to Bangkok, where they were examined by sugar flotation method. In the laboratory, 1 g of the sample was placed in a centrifuge tube with 10% sodium hypochlorite. The mixture was shaken to detach parasites from soil particles, and then centrifuged for five minutes at 2,000 rpm. After discarding the supernatant fluid and mixing the sediment with other media (sugar solution with a specific gravity of 1.200), the mixture was left at room temperature for two hours before microscopic examination [6].

Housefly samples

Houseflies were caught in a small restaurant and around households in the two villages. Each fly was covered with a single plastic bag and combined into one container for each catching site. These were then stored in a cooler box. Thirty flies were kept in a capped bottle containing 15 ml formalin-detergent (FD) solution (10 ml formalin and 50 ml detergent dissolved in 440 ml water), and sent to Mahidol University. In the laboratory, the preserved flies, in their preservative solution, were washed in an ultrasonic cleaner (Elma Transsonic Digital D-7700) at level five for 15 minutes at room temperature [7]. All sediment was observed under a light microscope for the presence of helminth eggs or larvae.

Vegetable samples

A total of 1,320 g of vegetable samples were taken, including some types frequently eaten raw (holy basil, hairy basil, spring onion, coriander, Chinese cabbage, lettuce, spiritweed/long coriander, kitchen mint, and heart leaf). These were collected from garden plots around the villages or purchased from the morning market. They were cut into small pieces and soaked in FD solution for 30 minutes, then washed in an ultrasonic cleaner at level five for 15 minutes at room temperature. The sediments were then examined under a light microscope for the presence of helminth eggs and larvae.

Results

Soil samples

Since our investigation was conducted during the raining season in Lao PDR, the soil samples collected were saturated with rainwater. One ascarid egg containing a degenerated larva was found in a sample collected near a septic tank in Ban Lahanam. In other soil samples, several unknown species of possible free-living nematode larvae were found.

Housefly samples

Houseflies were observed to be carrying opisthorchid eggs, taeniid eggs and mites on their body surfaces. The eggs of opisthorchid and taeniid worms were found either as a single egg or as a cluster of 10-20 eggs (Table 1). Unexpectedly, one sample of flies collected from the local restaurant at Ban Thakhamlaine village contained adult Haplorchis taichui worms, which were identified by their characteristic fan-shape spines on the ventral sucker (Fig 2) [8]. It is possible the presence of H. taichui on the body surface of these flies might have occurred after feeding on a duck's intestinal contents in the garbage. In rural areas here, food prepared from duck's flesh and its internal organs is very common.

Vegetable samples

In the vegetable samples collected (Fig 3), one strongylid worm egg was detected in a garden plot where cattle excreta were used as a fertilizer. The egg was oval, with a thin shell and contained a morula-stage embryo. Vegetables bought from the morning market were contaminated with nematode larvae and adults, as well as several mites and insects. Taeniid eggs were found in...
Table 1  Parasites found on the external body surfaces of houseflies in Ban Lahanam and Ban Thakhamlaine, Songkhone District, Savannakhet, Lao PDR, 10-12 August 2010.

<table>
<thead>
<tr>
<th>Site of collection</th>
<th>No. exam</th>
<th>No. of minute intestinal flukes</th>
<th>Taenia egg</th>
<th>Larvae**</th>
<th>Mites***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Around household</td>
<td>92</td>
<td>97, 2’</td>
<td>-</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Local restaurant</td>
<td>166</td>
<td>68, 1’</td>
<td>3 (H. taichui)</td>
<td>38</td>
<td>23</td>
</tr>
</tbody>
</table>

* cluster of eggs (each cluster contained 10-20 eggs); ** unknown species of nematode larvae; *** ectoparasites of mammals and avians

Table 2 Parasites discovered on vegetables collected from the village of Ban Thakhamlaine, 10-12 August 2010.

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount exam (g)</th>
<th>No. of parasites discovered</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>egg</td>
<td>larvae</td>
</tr>
<tr>
<td>Vegetable plot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>holy basil &amp; hairy basil</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local market</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spring onion &amp; coriander</td>
<td>335</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>holy basil</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>245</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lettuce</td>
<td>235</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spiritweed/long coriander</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kitchen mint</td>
<td>50</td>
<td></td>
<td>unknown</td>
</tr>
<tr>
<td>heart leaf</td>
<td>120</td>
<td></td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>unknown</td>
</tr>
</tbody>
</table>

* larvae with constriction at tail-end; E.v = Enterobius vermicularis; T.t = Trichuris trichiura

the spiritweed and kitchen mint. Oxyurid and trichurid eggs were detected in heart leaf, which is often eaten raw by the local populace. One trichostrongylid egg and nematode larvae were detected on spring onions and coriander bought from the local market (Table 2). Of the six
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Fig 2  *Haplorchis taichui* discovered on external body surface of houseflies: a) adult worm; b) ventral sucker with fan-shaped spines.

Fig 3  The examined vegetables: a) holy basil; b) hairy basil; c) spring onion and coriander (sold bunched together); d) Chinese cabbage; e) lettuce; f) spiritweed/long coriander; g) kitchen mint; h) heart leaf.
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Fig 4  *Angiostrongylus* sp 3rd stage larva isolated from vegetables: a) whole larva; b) well-developed chitinous rod in the buccal cavity; c) constriction at the tail-end.

Fig 5  Helminth eggs in the collected samples: a) *Ascaris* sp with degenerated larva; b) *Taenia* sp; c) Strongylid; d) *Trichostrongylus* sp; e) *Enterobius vermicularis*; f) *Trichuris trichiura*. 
larvae found, three had characteristics specific to third-stage Angiostrongylus larvae: a well-developed chitinous rod in the buccal cavity and a constricted tail (Fig 4). Morphologically, none of the recovered adult worms resembled any species of human or animal pathogenic nematode, so may be free-living nematodes in the soil.

Taeniid eggs contaminating the houseflies and soil samples might be Taenia or Echinococcus, since the study site is a ruminant-raising area.

**Discussion**

We confirmed that the soil, housefly and vegetable samples taken from the study villages were highly contaminated with helminthes (Tables 1, 2). This suggests a high risk of infection for villagers and animals in the local area. Angiostrongyliasis is a medically important helminthic disease, since it causes meningoencephalitis and other severe symptoms. Generally, the intermediate hosts of Angiostrongylus are animals, such as mollusks, freshwater prawns, terrestrial crabs, and frogs [9,10]. Vegetables have also been described as a possible source of angiostrongyliasis [11-14]. In this study, we were able to isolate the infectious larval stage of Angiostrongylus sp on vegetables bought from the local market. It is possible, therefore, that villagers are at risk of contracting angiostrongyliasis infections, not just from eating raw snail intermediate hosts, but by simply consuming raw vegetables. In these remote parts, populations are at high risk of Angiostrongylus infection, since they believe that home-grown vegetables are clean, chemical-free, and safe to eat raw, sometimes even without washing. This belief may increase the risk of infection in some areas. Surprisingly, houseflies were found to carry many parasitic objects. They were confirmed as having a role as a mechanical vector for helminthic infections, capable of mediating disease transmission within the community. Also, it has been reported that helminthic objects, protozoan cysts and bacteria are carried by houseflies from place to place, and from animal to human and vice versa, in many countries [15-19]. Flies are not just unpleasant pests to humans, but also medically significant animals that transmit infectious diseases.

In a previous study by Sato et al [3], the prevalence rate of Trichostrongylus infection in Ban Lahanam Village was rather high (21.2%), leading us to expect to find many infective-stage larvae in the environment. However, only one trichostrongyloid egg was found on the spring onions and coriander. As this study took place during the rainy season, many parasitic objects in the soil might have been washed away by the rain. The numbers found may increase if samples are taken and examined during the dry season. To confirm this hypothesis, further investigations should be conducted to compare levels of helminthic-object contamination in the environment at different times of the year. Environmental factors are very important when considering mechanisms of parasitic transmission, especially in times of unpredictable climate change and increasingly frequent natural disasters. In rural, parasitic-disease-endemic areas, humans and animals are surrounded by pathogens and share the same unsanitary environment. To prevent disease transmission, appropriate health education about sources and transmission methods of infection, as well as about safe food handling, preparation and storage, should be provided to local communities.

**Acknowledgements**

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**References**