Mixed Infection of Zoonotic Foodborne Trematodes and Intestinal Nematodes in a Community in Southern Lao PDR

การติดเชื้อร่วมระหว่างพยาธิใบไม้ติดต่อทางอาหารจากสัตว์สู่คนและพยาธิตัวกลมลำไส้ในชุมชนภาคใต้ประเทศลาว

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ABSTRACT

Southern part of Lao PDR is a known endemic area of foodborne zoonotic trematode including *Opisthorchis viverrini* but coexisting intestinal parasites are also present. This study aimed to examine a comprehensive pattern of endemic parasites in the community in Savannakhet Province, Lao PDR by using formalin-ethyl acetate concentration and agar plate culture technique for fecal examination. The results revealed that hookworm was predominant with 74% prevalence followed by *Opisthorchis*-like eggs or foodborne zoonotic trematodes (FZT) 62% and *Strongyloides stercoralis* 20%. The findings demonstrated that in addition to FZT, soil-transmitted nematodes are a significantly neglected tropical disease in Southeast Asia.

บทคัดย่อ

ทางตอนใต้ของประเทศสาธารณรัฐประชาธิปไตยประชาชนลาวเป็นพื้นที่ระบาดของพยาธิใบไม้ที่ติดต่อโดยการปนเปื้อนในอาหารจากสัตว์สู่คน รวมทั้งพยาธิใบไม้ตับ นอกจากนั้นยังพบว่ามีการติดเชื้อพยาธิตัวกลมในลำไส้ร่วมด้วย การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาให้ครอบคลุมการระบาดของปรสิตที่มีในชุมชนจังหวัดสวรรณเขต ประเทศสาธารณรัฐประชาธิปไตยประชาชนลาวโดยใช้วิธี formalin-ethyl acetate concentration และ agar plate culture ผลการตรวจอุจจาระพบการติดเชื้อพยาธิเรียงตามลำดับความชุกคือ พยาธิปากขอ (hookworm) ร้อยละ 74, *Opisthorchis*-like eggs หรือพยาธิที่ติดต่อโดยการปนเปื้อนในอาหารจากสัตว์สู่คน ร้อยละ 62 และ *Strongyloides. stercoralis* ร้อยละ 20 จากผลการศึกษานี้แสดงให้เห็นว่านอกจากพยาธิใบไม้ที่ติดต่อโดยการปนเปื้อนในอาหารจากสัตว์สู่คนแล้วยังมีพยาธิตัวกลมที่ติดต่อผ่านดินเป็นโรคเขตร้อนที่ถูกละเลยในภูมิภาคเอเชียตะวันออกเฉียงใต้

Key Words: Foodborne Zoonotic Trematodes, *Opisthorchis*-like eggs, Neglected tropical diseases
คำสำคัญ: พยาธิใบไม้ปนเปื้อนในอาหารติดต่อจากสัตว์สู่คน ไข่พยาธิที่ลักษณะคล้ายไข่พยาธิใบไม้ตับ โรคเขตร้อนที่ถูกละเลย

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Introduction

Foodborne zoonotic trematodes (FZT) and intestinal nematodes are common in tropical areas. Among FZT, the liver fluke, *Opisthorchisviverrini* is endemic in Southeast Asia, including Thailand, Lao People's Democratic Republic (Lao PDR), Vietnam and Cambodia (WHO, 1995; Chai et al., 2005). While infection with intestinal FZT causes mild pathology, infection with the liver fluke (opisthorchiasis) associated with hepatobiliary diseases, including cholangitis, obstructive jaundice, hepatomegaly, cholecystitis, cholelithiasis and cholangiocarcinoma (Elkins et al., 1990; Sithithaworn et al., 1994; Mairiang et al., 2012). Because of drug treatment, the prevalence and intensity of parasitic infection such as *O.viverrini* infection has been greatly reduced in several endemic areas in Thailand (Jongsuksuntigul & Imsomboon, 2003). In addition to current endemic situation of low prevalence and intensity, the nature of over dispersed distribution pattern of the parasite in humans and people with light infection are predominant in endemic communities. Therefore there is need for more sensitive and specific diagnostic tests as essential tools to support and facilitate a surveillance and control of parasitic diseases in this region.

Diagnosis of parasitic infection is typically based on conventional parasitological methods, such as formalin-ethyl acetate concentration technique (FECT) or Kato Katz technique (KK). But distinguishing opisthorchiid eggs from some FZT by morphological criterion alone is difficult under light microscopy because the shape and size of haplorchiidae and opisthorchiid fluke eggs are very similar (Giboda et al., 1991; Ditrich et al., 1992; Radomyos et al., 1998; Sukontason et al., 2001). Mixed infections with *O. viverrini*and minute intestinal flukes belonging to the Heterophyid (*Haplorchis taichui*, *Haplorchis yokogawai Haplorchis pumilio*, and *Echinochasmus japonicus*) and Lecithodendriidae (*Phaneropsolus bonnei and Prosthodendrium molenkampi*) families as well as soil transmitted nematodes are common and have been reported in Thailand and Lao PDR (Giboda et al., 1991; Radomyos et al., 1998; Sukontason et al., 2001; Chai et al., 2005; Waikagul & Radomyos, 2005). Among alternative diagnostic methods, these included serological tests based on enzyme-linked immunosorbent assay (ELISA) for antibody detection are useful under some situations but they cannot distinguish between recent or past infection are often faced with problems of cross reactivity with other parasites. Another immunodiagnostic method based on faecal antigen detection has been explored and is preferable since it may reflect current rather than past infection (Chaicumpa et al., 1992; Sirisinha et al., 1995). In addition, positive antigen detection may be found in egg negative cases of opisthorchiasis (Sirisinha et al., 1995). A drawback of the copro-antigen detection technique is that specific clone(s) of monoclonal antibody are required in the test and they are not always available. To date, conventional parasitological methods are considered as gold standard and though their reliabilities and accuracies are sometime questioned (Johansen et al., 2010).

In Lao PDR, several previous studies have shown that among FZT there are coexisting intestinal helminthes such as minute intestinal flukes (MIF), intestinal nematodes e.g. *Ascaris, trichuris*, hookworms and *Strongyloides* (Rim et al., 2003; Chai et al., 2005; Sithithaworn et al., 2006). By conventional diagnostic method such as formalin-ethyl acetate concentration technique (FECT), it is not possible to distinguish *O.viverrini* and some MIF particularly *Haplorchis spp*. Hence most studies from Lao PDR as well as Vietnam reported findings of *O.viverrini*-like or FZT egg and the actual prevalence of each group of parasite is not clear. In addition, coexisting intestinal nematode infection may be overlooked due to low sensitivity of the diagnostic method (Sithithaworn & Haswell-Elkins, 2003; Sithithaworn et al., 2005). Suitable methods for diagnosis of hookworms and *Strongyloide,* such as agar plate culture technique (APCT) are rarely used in community-based surveys.

Objectives of the study

In order to examine diversity of helminth parasite endemic in southern Lao PDR, human stool samples obtained from an endemic community in Savannakhet Province, Lao PDR were analyzed by using both FECT and APCT as diagnostic methods. Specifically, the objectives were to obtain current prevalence and intensity of FZT, hookworms, *Strongyloides stercoralis* and other parasite such that effective long term strategy for prevention and control can be designed and implemented.

Methodology

The sample population originated from a endemic community at Thatinghan villages in Savannakhet Province, Lao PDR. Fecal samples were originally collected by Ms Saycocie and coworkers for a study on status of parasitic infection and their relationship with eosinophilia and leukocytosis in an endemic community in Savannakhet Province, Lao PDR (Saycocie, 2007). The study protocol of this study was approved by the ethical research committee on human, Khon Kaen University (4.2.09: 7/2550).

Analyses of fecal samples were done by FECT following the protocol previously described (Elkins et al., 1990). By morphological criterion, *O.viverrini* is indistinguishable from *Haplorchis sp* (hence considered here as FZT) but can be differentiated from *Phaneropsolus bonnei* and *Prosthodendrium molenkampi* (Kaewkes et al., 1991). For FZT, the number of eggs per gram of feces (EPG) was calculated by the formula below.

EPG = nV/Vw

n = number of eggs

v = number of drops examined

V = total volume of sediment in drops

W= weigh of faeces in gram

For APCT, the technique is highly effective for coprologocal diagnosis of human strongyloiasis and the procedure employed here followed the description originally described (Koga et al., 1991; Sithithaworn & Haswell-Elkins, 2003). Essentially, the fecal specimen (4 g) was placed on the agar plate (1.5% agar), sealed the plate with parafilm and let alone for 2-4 days at room temperature. The plates were washed with 10% formalin and pipette to the centrifuge tube for examination of the parasites in the sediments. The characteristic morphology of various free-living stages of *S. stercoralis* as well as hookworms were identified and recorded.

Data obtained were kept in Excel and subsequently analyzed by SPSS. The distributions of data were assessed for their normality prior to statistical analysis and data transformations were performed when necessary. Statistical tests were considered significant when p-value was less than 0.05.

Results

Characteristics of sample population

Two hundred and thirty-four fecal specimens analyzed in this study were originally collected from people living in Savannakhet Province, Lao PDR. The age and sex distribution of the sampled subjects were shown in Table 1. The subject age ranged between 17 to 92 years and the overall mean of age was 44 years with 92 (39.3%) and 142 (60.7%) were males and females, respectively.

Table 1 Characteristics of subjects whose stool samples were recruited in this study (n = 234)

Age group	N	Sex	
		Male	Female
11-20	31	18	13
21-30	21	5	16
31-40	63	18	45
41-50	46	23	23
51-60	35	16	19
> 60	38	12	26
Total	234	92 (39.3%)	142 (60.7%)

Prevalence of parasitic infection

The prevalence of parasite infection as determined by FECT and APCT was shown in Table 2. The prevalence of infection within the sample population by order were hookworms (74.8%, 175 cases), *O. viverrini*-like egg (62.4%, 146 cases), *Strongyloides stercoralis* (20.9%, 49 cases), *Taenia spp*. (11.1%, 26 cases), *Ascaris lumbricoides* (10.7%, 25 cases), *Trichuris trichiura* (2.1%, 5 cases), Minute intestinal flukes (2.1%, 5 cases), *Enterobius vermicularis* (0.4%, 1 case) , Echinostomes spp (0.4%, 1 cases). The overall parasitic infection rate was 87.6% and mixed infection with more than one species of parasite was 63.2%.

Analyses of infections by sex revealed that there were slightly more infection in females than males for *O.viverrini*-like infection, hookworms and *Strongyloides* while there more infection in male for *Ascaris* (Figure 1).

Table 2 Prevalence of parasitic infection within the sample population determined by FECT and APCT (n = 234)

Parasite	n (%)
Hookworm spp.*	175 (74.8)
Opisthorchis viverrini-like egg	146 (62.4)
*Strongyroides stercoralis**	49 (20.9)
Taenia spp.	26 (11.1)
Ascaris lumbricoides	25 (10.7)
Trichuris trichiura	5 (2.1)
Phaneropsolus bonnei	4 (1.7)
Prosthodendrium molenkampi	1 (0.4)
Enterobius vermicularis	1 (0.4)
Echinostome spp.	1 (0.4)
Single species infection	57 (24.4)
Multiple species infection (2-6 species)	148 (63.2)
Total parasitic infection	205 (87.6)

*Hookworm spp and *S. stercoralis* infection were combined from APCT and FECT

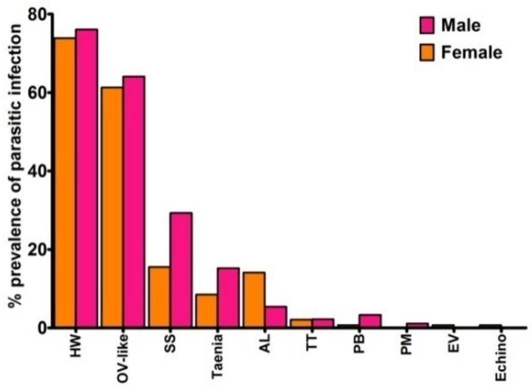

Figure 1 Sex-related prevalence of parasitic infection within the sample population determined by FECT (n = 234). HW= Hookworm spp, OV-like = *O. viverrini* like egg or FZT, SS = *Strongyloides stercolaris*, Taenia = Taenia spp, AL=*Ascaris lumbricoides,* TT= *Trichuris trichiura,* PB = *Phaneropsolus bonnei*, PM= *Prosthodendrium molenkampi,* EV= *Enterobius vermicularis and* Echino=Echinostomes. Hookworm spp and *S. stercoralis* infection were combined from APCT and FECT

Age-sex prevalence and intensity of *O.viverrini*-liked or FZT infection

The prevalence of infection peaked at age group 31-40 years and then declined as age increased while the intensity were similar between age groups and in males and females (Figure 2).

The frequency distribution of *O. viverrini*-like infection classified according to egg per gram faeces (EPG) were divided in 4 groups (Table 3). The first group was egg negative, group 2 was 1-500 EPG, group 3 was 501-1500 EPG and the last group was greater than 1500 EPG. The frequency distribution and mean EPG in each intensity class were shown in Table 3. The mean EPG of egg-positive sample was 667.5 EPG. Range of OV-like eggs were 7-24,004.

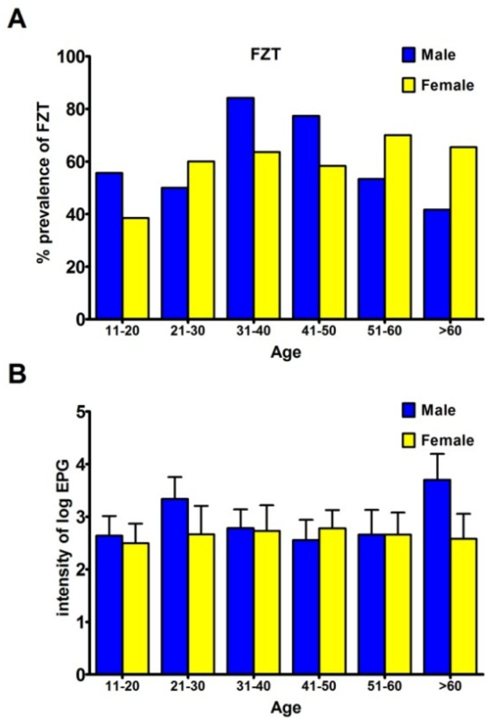

Figure 2 Age-sex prevalence of food borne zoonotic trematode (A) and intensity of infection (B) in the sample population from Savanakhet Province grouped according to age and sex of subject

Table 3 Frequency distribution of subjects grouped according to intensity of *O. viverrini*-like infection (EPG) and the average, standard deviation and range of infection intensity

Group	No.	Intensity	Mean EPG (±SD)	Range (Min-Max)
1	88	0	0	0
2	108	1-500	103.1 (±117.4)	7-479
3	22	501-1500	911.5 (±349.5)	504-1484
4	16	>1500	4142.1 (±5444.8)	1674-24006
Total	234		667.5 (±2161.7)	

Prevalence and intensity of hookworms

The prevalence of hookworms had no apparent relation with age and males had slightly more prevalence than females (Figure 3). Similarly the intensity of infection showed an age-related pattern with significantly lower intensity in the group of 21-30 year ($p<0.05$).

Frequency distribution of hookworms had a typical over dispersion pattern with high intensity (epg>1500) occurring in 10 out of 234 individuals (4.2%) (Table 4).

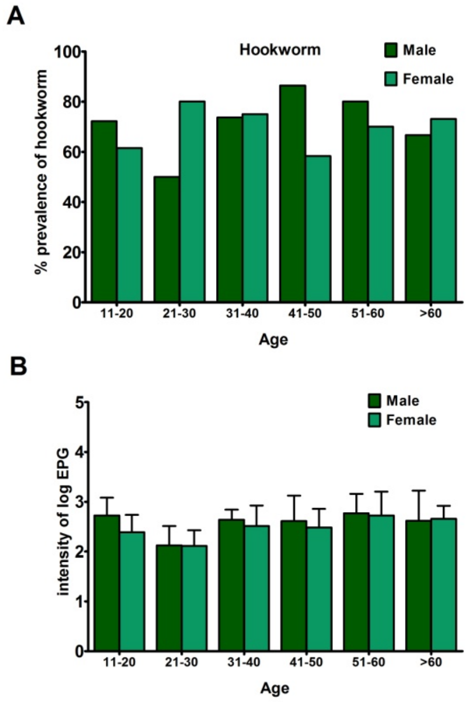

Figure 3 Age-sex prevalence of hookworms (A) and intensity of infection (B) in the sample population from Savanakhet Province grouped according to age and sex of subjects

Table 4 Frequency distribution of subjects grouped according to intensity of hookworm infection (EPG) and the average, standard deviation and range of infection intensity

Group	No.	Intensity	Mean EPG (±SD)	Range (Min-Max)
1	65	0	0	0
2	131	1-500	153.2 (±137.4)	8-494
3	28	501-1500	852.0 (±259.5)	542-1484
4	10	>1500	2240.6 (±783.1)	1560-4142
Total	234		392.5 (±584.3)	

Prevalence and intensity of *Strongyloides stercoralis*

Similar to hookworms, the prevalence of *S.stercoralis* changed varied very little with age (Figure 4). Males had significantly higher prevalence than females ($p<0.05$). There was no pattern of age-related intensity of infection (data not shown).

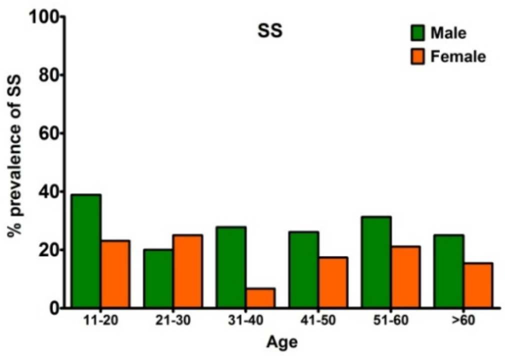

Figure 4 Age-sex prevalence of *S. stercolaris* in the sample population from Savanakhet Province as determined by FECT and APCT

Discussion and Conclusions

Control of neglected tropical diseases requires appropriate diagnostic tests and their efficacies depend on the purpose of the control program (Bergquist et al., 2009). In a period of post chemotherapy or surveillance, high specificity and sensitivity methods other than the conventional diagnosis is needed. By using FECT and APCT, the result observed in this study were quite different from others. Hookworm was the most common helminth with prevalence of 74.8% followed by *O.viverrini*-like eggs or FZT (62.4%). *Stronyloides* (20%) was the second most common intestinal nematode parasite while *Ascaris*and *Taenia* had similar prevalence of infection (10-11%). Within the *O.viverrini*-like eggs, there could be *O.viverrini* and other minute intestinal flukes similar to those reported earlier (Chai et al., 2009). Regarding the intensity of *O.viverrini*-egg infection, the distribution pattern was similar to other helminths for the over dispersed pattern. The trends for age-sex related prevalence for hookworms and *O.viverrini*-like eggs were almost the same in all age groups. These patterns indicated persistent exposure to infection and extensive transmission in the study area. The results from this study also indicated that by using APCT, hookworms were discovered as the most common parasite other than *O.viverrini*-like or FZT. Mixed infection between either hookworms or other intestinal nematodes such as *S.stercoralis*is common in this part of the world. Previous large survey in Lao PDR using Kato-thick smear method revealed that the cumulative egg positive rate for intestinal helminths was 61.9% and *Ascaris lumbricoides* was 34.9%, hookworm 19.1%, *Trichuris trichiura* 25.8%, *Opisthorchis viverrini* 10.9% (Rim et al., 2003). Another earlier study in Lao PDR also reported relatively lower prevalence of hookworm (28%) and *S. stercoralis* (19%) even using APCT for diagnosis (Vannachone et al., 1998). Thus apart from endemicity level, the diagnostic method also influenced transmission rates of the parasites i.e. FZT and intestinal nematodes.

For multiple drug treatment for example using praziquantel and albendazole there is need to carefully monitor their efficacies. In particular, treatment of *S. stercoralis* required special attention to avoid complication in immunocompromised individuals and that a 5 day course of albendazole is recommended and repeated doses may be needed in cases with treatment failure (Suputtamongkol et al., 2011). In addition, unlike a single dose treatment as for praziquantel, a 5 daily dose treatments for albendazole is at risk of low compliance in the field and poses problems for control.

The findings of high prevalence of hookworms and also OV-like egg or FZT are not unexpected in this part of Southeast Asia. The question remains, however, to confirm the actual proportion of *O.viverrini* eggs among FZT. Since *O.viverrini* is a carcinogenic liver fluke and may cause serious cancer later on in life, it is important to accurately measure its prevalence so that curative treatment can be given. Separation and identification of *O.viverrini* eggs requires molecular or immunological diagnostic procedures (Johansen et al., 2010) which may not be practical in the point of care setting or remote community. Therefore, a simplified, economic priced test kit for detection of *O.viverrini* within FZT-positive individuals is still needed. Conventional methods such as FECT and APCT are still needed as basic tools prior to a more modern technique to combat neglected tropical diseases in Southeast Asia and other parts of the world.

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