EPIDEMIOLOGY OF FOOD-BORNE TREMATODES AND OTHER PARASITE INFECTIONS IN A FISHING COMMUNITY ON THE NAM NGUM RESERVOIR, LAO PDR

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Abstract. Food-borne trematodes (FBT) are important causes of parasitic infections in many Asian countries. Parasitological surveys in Xai Udom, a small fishing community on the Nam Ngum reservoir, Lao People's Democratic Republic, revealed an overall parasitic infection rate in May 1999 of 68.8% (n=173) and in December 1999 of 65.9% (n=261). The liver fluke, *Opisthorchis viverrini* accounted for most of the infections (prevalences of 53.8% and 42.1%, during the first and second surveys, respectively). The prevalence and intensity showed increasing trends with age. Minute intestinal flukes were also present but with relatively low infection rates (3.8-10.9%). The second common group of parasites comprised soil-transmitted nematodes, *Trichuris trichiura*, *Ascaris lumbricoides*, hookworm and *Strongyloides stercoralis,* with prevalences of 22.4 and 17.6%, 20.8 and 8.0%, 16.8 and 13.4%, and 4.0 and 15.3% (first and second surveys, respectively). Most people had no or only light infections, with a few people having heavy infections. Coexisting intestinal protozoa were *Giardia lamblia* (5.2 and 4.9%) and *Entamoeba coli* (6.9 and 6.5%). Concurrent tapeworm infections were *Taenia* (1.7 and 1.1%) and *Hymenolepis nana* (0.7 and 0.6%) (first and second surveys, respectively). Despite the availability of effective anthelmintic drugs, the results of our study reaffirm that FBT are still a major health threat in this fishing area of Lao PDR, similar to a decade ago.

INTRODUCTION

The liver fluke, *Opisthorchis viverrini*, is the most important food borne trematode (FBT) in Southeast Asia with respect to the number of infected people and the associated morbidity (WHO, 1995). The North and northeastern parts of Thailand, adjacent to the Lao People's Democratic Republic (PDR), have been a major focus of infection. The baseline patterns of parasite distribution are well-documented (Jongsuksuntigul and Imsomboon, 2003; Sithithaworn and Haswell-Elkins, 2003). In northeastern Thailand, the prevalence and intensity of liver fluke infection correlates with

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the incidence of cholangiocarcinoma (cancer of the biliary tract) (Vatanasapt *et al,* 1990; Sithithaworn *et al,* 1994; Sriamporn *et al,* 2004). While information on the distribution of *O. viverrini* in other endemic countries, such as Cambodia and Vietnam, is limited, evidence for the infection in Lao PDR was available almost a decade ago, notably in communities around Nam Ngum reservoir, in the Keoudom and Thulakhom districts of Vientiane (Giboda *et al,* 1991a,b). Fish collected from the Num Ngum reservoir and other areas in Vientiane were also reported to harbor several types of trematode metacercariae, including *O. viverrini* (Ditrich *et al,* 1990). In addition to Vientiane, the infection was reported in Khummoune (Kobayashi *et al,* 1996; Vannachone *et al,* 1998; Kobayashi *et al,* 2000) and Chumpasak Provinces (Chai and Hongvanthong, 1998). A recent nation-wide survey of primary school children showed an extensive distribution of the parasite in the country and a high prevalence was found in Khummoune and other southern regions, particularly those along the Mekong River (Rim *et al,* 2003).

This study was a part of a collaborative project between the Food and Agriculture Organization (FAO) and the Lao PDR Government on improved utilization, quality and safety of fish and fishery products. The target area was around the Nam Ngum reservoir, the main fishing area in the Lao PDR. This study aimed to determine the current epidemiological patterns of parasitic infection in a fishing community in the Nam Ngum area.

MATERIALS AND METHODS

Description of the study area and sample population

Xai Udom was selected for this study based on the presence of fish processing activities and the availability of fishery products. Evidence for the presence of FBT infection in this community led the local health officer to carry out a mass treatment program in 1995, but no documented record of that was available. Geographically, Xai Udom is one of several fishing communities locating around the Nam Ngum reservoir. This community consists of 126 households with a total population of 779, of whom approximately 70% are fishermen. The estimated daily catch is 1,400 kg. The general sanitary conditions in the village are poor, as only a single well with underground water is available for community use and a few simple pitted latrines were observed. There is one health station in the village with one health officer who is responsible for any existing health problems. No electricity for household use was available at the time of the project, but a few electric solar cells are now being installed by a charity organization. The main income of the villagers is from fishing in the reservoir.

Sample collection and parasite examination

With the collaboration of local staff from the Ministry of Health, fecal samples were collected from villagers during May 1999 and December 1999. The sample subjects were recruited from the villagers by a random household sampling method to assess the prevalence and intensity of parasitic infection. In the first visit in May 1999, 300 stool containers were distributed to 50 households and 171 fecal samples were returned, giving a compliance rate of 57%. In the second visit in December 1999, 500 stool containers were handed to 100 households and 261 specimens were obtained, thus the compliance rate was 54.2%. The age and sex of cooperating individuals were noted. Two grams of individual fecal samples were weighed and transferred to test tubes, and fixed with 10% formalin in the field. The formalin-fixed specimens were further processed by quantitative formalin-ethyl acetate concentration technique and examined for parasites in the laboratory as previously described (Elkins *et al,* 1990). Parasite-positive individuals were treated with ap-

propriate anti-parasitic drugs at the end of the study.

Examination of fish for trematode metacercariae

Cyprinoid fish from near the village and those available at the main landing site at Nam Ngum Dam were sampled to assess the presence of trematode metacercariae as possible sources of FBT infection. The fish samples were placed on ice and taken to the laboratory in Vientiane. At the laboratory, they were sorted according to species, counted and weighed prior to determining the metacercariae. The pepsin digestion method (Sithithaworn *et al,* 1997) was employed for this purpose. Briefly, a sample of each species of fish was minced in 0.625% pepsin (BDH) using a commercial blender and the homogenate incubated at 37°C for 1 hour with frequent stirring. The digest was then filtered through a series of meal sieves and the filtrate concentrated in a sedimentation jar with several washes of 0.9 % NaCl (NSS). The final sediment was examined under a dissecting microscope and the metacercariae collected and identified.

Statistical analyses

Comparisons of the percent prevalence and variation of prevalence with age were assessed by the χ^2-test. Due to the distribution of the data for intensity of infection, statistical analyses on variation of intensity of infection by age were performed using non-parametric analysis of variance. $p \leq 0.5$ was considered significant. The statistical software used was SPSS version 10.

RESULTS

Rates of parasitic infections

As shown in Table 1, the list of parasites endemic in the study area consisted of food borne helminths, soil transmitted nematodes and water/food-borne intestinal protozoa. The overall prevalence rates of parasitic infection

Table 1
Prevalence of parasitic infections estimated by stool examination based on the formalin-ethyl acetate concentration technique in a fishing community in Xai Udom. Data shown are number and percent of infected individuals.

Parasites	Number infected (%)	Number infected (%)
Date of sampling	May 1999	December 1999
N	173	261
Opisthorchis viverrini	93 (53.8)	110 (42.1)
Minute intestinal flukes	19 (10.9)	10 (3.8)
Echinostomes	1 (0.6)	-
Taenia sp	3 (1.7)	3 (1.1)
Hymenolepis nana	1 (0.6)	2 (0.7)
Capillaria philippinensis	1 (0.6)	-
Hookworm	29 (16.8)	35 (13.4)
Strongyloides stercoralis	7 (4)	40 (15.3)
Ascaris lumbricoides	36 (20.8)	21 (8)
Trichuris trichiura	39 (22.5)	46 (17.6)
Giardia lamblia	9 (5.2)	13 (4.9)
Iodamoeba butschlii	1 (0.6)	-
Entamoeba coli	12 (6.9)	17 (6.5)
Total	119 (68.8)	172 (65.9)

observed in both surveys were 68.8% in May and 65.9% in December 1999. Among the food borne trematodes, the majority were the liver fluke, *O. viverrini*, occurring in 53.8% and 42.1% of the sampled populations examined on the first and second visits, respectively. Minute intestinal flukes (MIF) were also present in low percentages (3.8 and 10.9%). Echinostomes and tape worms were also detected in a few individuals. *Capillaria philippinensis* was detected in a 49-year-old female in May 1999.

The soil transmitted nematodes *A. lumbricoides*, *T. trichiura*, hookworm and *S. stercoralis* were also present in this community with prevalence rates ranging from 4 to 22.5%. *A. lumbricoides* and *T. trichiura* were the most commom with prevalences of 17.6 and 22.5%. For intestinal protozoa, *G. lamblia* and *Entamoeba coli* were consistently observed at both sampling times with prevalence rates of 4.9 and 6.9%.

Age-prevalence and intensity of liver fluke infection

The profiles of age-related prevalence and intensity of *O. viverrini* infection are presented in Fig 1. The prevalences of infection showed a rapid raise with age, stabilizing at 21-30 years, then peaking at ages 41-60 years (69-100%). The changes in prevalence rates were significantly associated with age in both surveys (χ^2-test, $p<0.001$). By contrast, the intensity of infection measured by eggs per gram (epg) of feces showed gradually increasing trends (Kruskal-Wallis test, $p<0.001$) with the highest intensity appearing in the oldest age group (>60 years) who had the longest exposure to infection. The youngest age found infected was 2 years. The prevalences in male and females were 48.6% and 51.4% in May and 47.5% and 53.3% in December, respectively. The average intensities of infection in males and females were 96.6 and 79 epg in May and 122.9 and 97.2 epg in December, respectively. No significant differences in

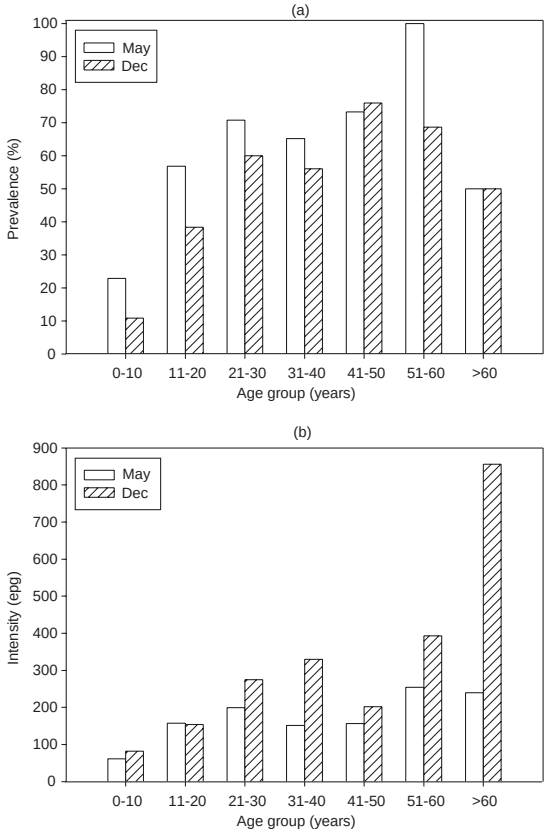

Fig 1–Age-related prevalence (a) and intensity profile (b) of *O. viverrini* in the sample population in Xai Udom in May and December 1999.

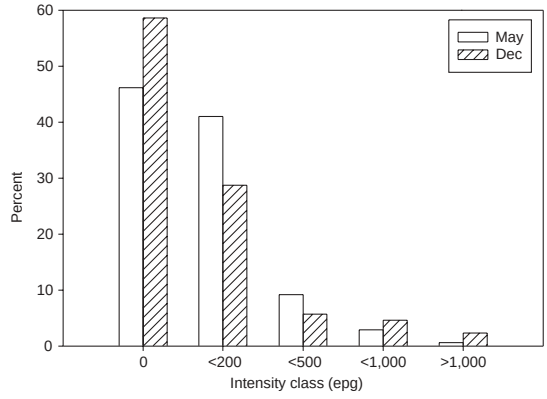

Fig 2–Distribution of *O. viverrini* in the sample population of Xai Udom village in May and December 1999. Data shown are percent of individual and intensity class (epg indicates eggs per gram feces).

prevalences or intensities between sexes were observed.

Fish surveys for trematode metacercariae

Among the 4 species of cyprinid fish analysed, *Cyclocheilichthys apogon* (n=7) were found to harbor *O. viverrini* metacercariae the 3 other species of fish, namely *Puntius orphoides* (n=31), *C. apogon* (n=8) and *Hampala dispar* (n=4) had both *O. viverrini* and *Haplorchis* sp metacercariae. No metacercaria was found in *C. armatus* (n=12).

Frequency and distribution of infection

Fecal egg output (in epg) for most of the helminth parasites observed in this study were found to be an accurate estimate of worm burden. Information on the distribution of intensity of infection within the sample population is useful for predicting worm burden and associated morbidity. Fig 2 depicts the distribution pattern of *O. viverrini*, which highlights the fact that the majority of infected individuals, (28.7% in May and 41% in December), fell within the light intensity class (<200 epg), while a smaller proportion of the sample (12.6-13%) had higher worm loads (>200 epg). Only 3.5% of individuals in May and 6.9 % in December had >1,000 epg. The greatest infection in May was 1,067 epg and in December was 2,115 epg.

Similar patterns of distribution were seen for *A. lumbricoides*, *T. trichiura, S. stercoralis* and hookworm. Most infected individuals showed a low intensity of infection, while a small percentage of the population had moderate to high worm burdens (data not shown).

DISCUSSION

In Lao PDR, detailed information regarding the prevalence of specific FBTs and their impact on human health in the major fishing area in Num Ngum reservoir was available as early as 1990 (Giboda *et al,* 1991a). Since then, there has been no published follow-up. Nevertheless, anthelmintic treatment has been provided to infected individuals in rural communities, although this did not occur on a regular basis. The actual number of people treated and the outcome of such treatment have not been systematically recorded. Thus, the impact of parasite treatment at the community level is probably limited. Therefore, information regarding the species of parasite, their geographical distribution, and the severity of infection, is needed.

The overall rates of parasite infection in the sample population in Xai Udom obtained in this study, 65-68%, were not unexpected, since at the time of sampling there was no large scale control program being implemented. More important is the finding that liver fluke infection is still the most common infection in the fishing villages around the Nam Ngum reservoir (prevalence 42-52%). The infection rates are similar to those reported in the previous decade by Giboda *et al* (1991a). Additional endemic areas in southern Lao PDR in Khammoune and Champasak Provinces (Kobayashi *et al,* 1996, 2000) have demonstrated that liver fluke infection in Lao PDR is widespread in the general population, corresponding to the neighboring area of northeast Thailand.

The presence of metacercariae of both liver fluke and *Haplorchis* sp in cyprinoid fish encountered in this study indicates that freshwater fish are a probable source of FBT infection in the community, which is also supported by results from a previous report (Ditrich *et al,* 1990). One of the common fish preparations observed in Xai Udom was raw fish paste (pla som) as a source of FBT infection. This household processed fish product is distributed and sold in the community. In this study, we employed a qualitative digestion technique for metacercarial detection, thus the prevalence and metacercarial load of each individual fish was not known. Four out of five species of cyprinoid fish were found to harbor liver fluke metacercariae, while three out of the five species of fish had metacercariae of *Haplorchis* sp.

An in-depth study is needed to identify the roles of individual fish species in FBT transmission in this area, and to assess the influence of seasonal factors on the abundance of metacercariae (Vichasri *et al,* 1982; Sithithaworn *et al,* 1997). This information has important implications for parasite control, particularly for the optimal timing of chemotherapy (Hinz *et al,* 1994). It can also be taken into the Hazard Analysis Critical Control Point (HACCP) as a means for health education and food safety (WHO, 1995).

The morbidity related to hepatobiliary diseases and cholangiocarcinoma associated with liver fluke infection is associated with several factors, such as intensity of infection, age and sex of the infected individual (Elkins *et al,* 1990; Mairiang *et al,* 1992; Haswell-Elkins *et al,* 1994). Of these factors, intensity of infection is the most important determinant of hepatobiliary morbidity as assessed by ultrasound, with the severity of gall bladder abnormalities being reversed after praziquantel treatment (Mairiang *et al,* 1993). The age-related prevalence and intensity profiles of liver fluke infection observed in this study generally resemble those found in other areas of Lao PDR (Kobayashi *et al,* 2000). The relatively high prevalence rates occurring in children (<10 years) are similar to those found in other areas (51% in the 6-10 year age group) reported by Kobayashi *et al* (1996) and more recently by Rim *et al* (2003) where school children had prevalence rates of up to 32%. This is a reflection of the poor economic status and poor hygiene conditions of these communities.

Such high prevalence rates in young age groups suggest that the acquisition of infection occurs early in life. An infected individual may carry worms in the bile duct for a long time due to the worms long life span. This may initiate the rapid development of hepatobiliary disease. Thus, as an immediate goal, parasite control by chemotherapy is essential to reduce the risk of developing hepatobiliary disease and cholangiocarcinoma. However, reinfection is known to occur after treatment (Kobayashi *et al,* 2000) and a repeated treatment strategy is required. The possibility of predisposition to infection with *O. viverrini* in individuals who had previous heavy infections has been reported (Upatham *et al,* 1988). Therefore, targeted treatment of this particular group should be a priority for long-term control. In contrast to soil transmitted nematodes, strong parasite pathogenicity argues for treatment of all infected individuals regardless of intensity of infection.

Overdispersed distribution is common for helminth parasites (Anderson and May, 1983). Heavy infections with liver flukes are common in a small fraction of individuals, while the majority of people have a low worm burden (Haswell-Elkins *et al,* 1991). An autopsy study, which provided more accurate worm recovery data, confirmed the same pattern of distribution (Sithithaworn *et al,* 1991). Since fecal egg count is proportional to worm burden, the observed distribution of epg in this study suggests a similar distribution pattern to previous studies in Thailand (Jongsuksuntigul and Imsomboon, 1998). The implication is that this distribution enhances the possibility of under diagnosis in the low worm burden group (Sithithaworn *et al,* 1991), demanding a highly sensitive fecal examination technique to accurately detect the majority of lightly infected individuals. Moreover, parasite control by chemotherapy will move more people into this lightly infected category. As employed in this study for the systematic evaluation or surveillance of parasitic infection, the modified formalin-ethyl acetate concentration technique is recommended. The technique not only allows easier separation of liver flukes from intestinal flukes which often coexist within a given community, but allows a longer duration of storage for fecal samples. However, for routine purposes the less complicated methods, such as the Kato-thick smear or simple smear, are preferred.

The presence of soil transmitted nematodes, including *A. lumbricoides*, *T. trichiura*, hookworm and *S. stercoralis* in Xai Udom are similar to other studies in Lao PDR (Kobayashi *et al,* 1996, 2000; Vannachone *et al,* 1998). Our inspection of the village revealed there was inadequate clean water and most of the few toilets were in poor condition. This condition along with poor personal hygiene clearly favors the transmission of these nematodes as well as intestinal protozoa. Attempts to address the problem of soil transmitted nematodes have suggested that a combination of chemotherapy and other measures is essential for reducing reinfection rates (Siharath *et al,* 2000).

From this study, apart from FBT which are considered a major hazard in the area, the presence of a fish-borne nematode, *C. philipinensis*, in a human was also detected for the first time in Lao PDR. The infected subject was originally from Vientiane and had migrated to Xai Udom more than 20 years previously. However, because of the sporadic nature of *C. philippinensis* infection, the source of infection remains to be determined.

In contrast to malaria, there is currently no systematic control program for FBT in this rural community, although a member of the general public may obtain treatment if he or she travels to a hospital. In our opinion, selective case detection and treatment may benefit the individual but has little impact on parasite control since the percent coverage is very small and most individuals receiving treatment are urban inhabitants. Therefore, a multisector approach for parasite control, including treatment and health education with additional HACCP-based principles for food safety, should be considered in the strategy for the control and prevention of infection and avoidable morbidity. As long as the socioeconomic conditions and way of life of the people remain unchanged, integration of food safety measures to raise the level of awareness of FBT infection and improvement of sanitary conditions should be included in programs for parasite control.

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