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Brief communication (original)

Opisthorchis viverrini infection in minute intestinal fluke endemic areas of Chiang Mai Province, Thailand

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Background: Food-born trematode infections such as opisthochiasis are major causes of morbidity in Asia. Fish-borne trematode infections are commonly found in the northern and northeastern regions of Thailand. Several species of cyprinoid freshwater fish have been reported as secondary intermediate hosts.

Objectives: To determine the prevalence of infections with *Opisthorchis viverrini* and other intestinal parasites in Chiang Mai Province, Thailand, which is an endemic area for minute intestinal fluke infections in Northern Thailand due to environmental characteristics and the traditional consumption of raw fish.

Results: Stool samples were collected from 327 subjects from Cho Lae sub-district, Ban Pao sub-district, Mae Taeng district, Luang Nuea sub-district, Doi Saket district, and Nong Yaeng sub-district San Sai district, Chiang Mai. The subject ages were between 10 and 60 years. Of these, 144 were males and 183 were females (male to female ratio 1:1.27).

Conclusion: Parasites were found in 36 stool samples (11.0%). The majority of detected parasites (17 cases, 5.2 percent) were *Opisthorchis viverrini*. Other intestinal parasites included hook-worms, *Trichuris trichiura*, and minute intestinal flukes. The highest prevalence (31.2%) was found in Cho Lae sub-district, Mae Taeng district. The prevalence of infection was significantly associated with the health behaviour of the subjects. There was no significant association with sex-distribution.

Keywords: Liver fluke, minute intestional fluke, *Opisthorchis viverrini*.

Opisthochiasis caused by *Opisthorchis viverrini* remains a major public health problem in many parts of Southeast Asia including Thailand, Lao PDR, Vietnam, and Cambodia. The epicenter of this disease is located in Northeastern Thailand where high prevalence coexists with a high incidence of cholangiosarcoma: a major primary carcinoma of the liver with a very poor prognosis [1]. An acute infection with *O. viverrini* has usually no or few clinical manifestations. However, a case-control study was performed with 103 cases of cholangiosarcoma that showed at least two-thirds were attributable to *O. viverrini* [2]. Further studies in endemic regions led to the suggestion that besides the fluke, chemical carcinogens are involved as a cofactor. For example, increased intake of dimethylnitrosamine produced by bacteria in salted fish, consequent upon fluke infestation, is thought to contribute to carcinogenesis [3]. Many factors influence the survival and transmission rates of parasites. In the case of opisthorchiasis, the consumption of raw or undercooked fish is the primary vector of infection [4]. Unsanitary latrines and the prevalence of both intermediate host, freshwater snails and fish, are further factors that contribute to high rates of infection especially in Northeast and North Thailand [5, 6]. In the northeast, several types of preparations contain uncooked fish [7]. Of those, *Koi pla* (raw fish flesh chopped with garlic, lemon juice, fish sauce, chili, roasted ground rice and vegetables) is probably the

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most infective, followed by fish preserved for fewer than seven days, then *pla ra* and *jaewbhong*, in which viable metacercariae are rare [8,9]. Based on a regional survey in 1981, the overall prevalence of opisthochiasis in north eastern Thailand was as high as 34.6% [5]. As a consequence, the Ministry of Public Health started in 1984 a liver fluke control program, which was expanded in 1988 to cover all 19 north-eastern provinces. The main strategies for this control comprised three interrelated approaches, namely stool examinations and treatment of positive cases with praziquantel for eliminating human host reservoir, health education for promotion of cooked fish consumption to prevent infection, and the improvement of hygienic defecation for the interruption of disease transmission. As a result, the annual positive rate subsequently decreased to 9.4% in the year 2001 [10].

However, studies on post-treatment re-infection in the northeast demonstrated that populations with high pre-treatment intensity tended to have the highest intensity of re-infection (Upatham *et al*, 1988; Sornmani *et al*, 1984). Rapid re-infection despite treatment might suggest the absence of protective immunity and that some communities are predisposed to heavy infection probably due to their unchanged social habits. Besides consumption of raw or undercooked fish, contaminated utensils, unwashed hands, and food preparation surfaces are believed to have an important impact on the transmission of *O. viverrini*. However, most published descriptions of social habits regarding consumption of raw fish are anecdotal and careful sociological investigations are required [9].

Natural reservoirs have been reported as a source of trematode infection in human, especially in the northern part of Thailand. Mae Ngad Reservoir is located in Mae Tang district, about 60 km north of Chiang Mai, Mae Kuang Udomtara Reservoir is located in Doi Saket district, about 40 km south of Chiang Mai, Thailand. They were constructed to supply electricity and serves as an important water supply for agriculture and fisheries as well as flood prevention during the rainy season. They have many aquatic animals, especially cyprinoid fish, which serve as the intermediate hosts of trematode parasites. The present study was performed to determine health behavior that might be associated with *Opisthorchis viverrini* infection in Chiang Mai Province. This study was thought to provide useful data about health status and risk behavior that might be targets for future interventions.

Materials and methods

This cross-sectional survey was conducted in Cho Lae sub-district (47Q 0501349 UTM 2116898), Ban Pao sub-district (47Q 0502440 UTM 2118294), Mae Taeng district, Luang Nuea sub-district (47Q 0515669 UTM 2090749), Doi Saket district, and Nong Yaeng sub-district (47Q 0512933 UTM 2088353) San Sai district, Chiang Mai, during the month of January 2007. Investigators visited four randomly selected areas of Chiang Mai, accompanied by local Public Health Department officials. Subjects of all age-groups were assigned to the study-population after informed consent was given. All subjects were interviewed and stool specimens were collected. Stool specimens were fixed with formalin and carefully stored before examination in a laboratory at the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University. The formalin-ether concentration technique was used to process all specimens. The presence of intestinal parasite eggs, larva, and protozoa was determined microscopically.

Statistical data were analyzed using the software EPI-INFO (Version 2, Centers for Disease Control and Prevention, Atlanta, USA). Descriptive statistics were used to describe the distribution of the demographic and socio-economic characteristics of the subjects. The chi-square test was used to compare differences in the distribution of categorical variables, or the chi-square test for trend when appropriate. A statistically significant difference was determined when p-value was less then 0.05.

Results

Of an overall population of 20,263, 327 subjects were recruited including all age-groups from below 10 years of age to more than 60 years. Of these, 144 were male and 183 were females (male to female ratio 1:1.27). Further demographic data are shown in **Table 1**.

Microscopy revealed parasites in 36 stool samples (11.0%). The highest prevalence (18.2 percent) was found in villagers aged 41-50 years. The majority of detected parasites (17 cases, 5.2%) were *Opisthorchis viverrini*. Other intestinal parasites included *Trichuris trichiura*, hook-worm, and minute intestional fluke as is shown in **Table 2**.

Table 1. Potential factors associated with infections.

Factors	Examined	Total infected (%)
Sex	327	36 (11.0)
Male	144	21 (14.6)
Female	183	15 (8.2)
Status	315	36 (11.4)
Family head	125	15 (12.0)
House wife	121	12 (9.9)
Child	57	2 (3.5)
Other	12	2 (16.7)
Age	307	
< 10	5	0 (0.0)
10 - 20	27	1 (3.7)
21 - 30	20	2 (10.0)
31 - 40	56	2 (3.6)
41 - 50	88	16 (18.2)
51 - 60	76	8 (10.5)
> 60	35	1 (2.9)
Education	304	
Illiterate	17	2 (11.7)
Elementary school	213	23 (10.8)
Secondary school	37	1 (2.7)
Vocational	23	3 (13.0)
Bachelor's Degree	14	0 (0.0)
Higher degrees	0	0 (0.0)
Occupation	276	
Farmer	34	2 (5.9)
Gardener	64	4 (6.2)
Trade	21	0 (0.0)
Labor	113	20 (17.7)
Government employee	16	3 (18.7)
Other	29	1 (3.4)
Emolument (Baht)	275	
< 3,000	99	11 (11.1)
3,001 - 5,000	102	10 (9.8)
5,001 - 7,000	23	2 (8.7)
7,001 - 9,000	18	2 (11.1)
9,001 - 11,000	14	1 (7.1)
> 11,000	19	2 (10.5)

Table 2. Parasite infection rates in four areas of Chiang Mai Province.

District	Sub-district	Male		Female		Total	
		Number examined	*Number infected (%)*	*Number examined*	*Number infected (%)*	*Number examined*	*Number infected (%)*
Doi Saket	Luang Nuea	36	1 (2.8)	51	4 (7.8)	87	5 (5.7)
Mae Taeng	Ban Pao	32	1 (3.1)	38	0 (0.0)	70	1 (1.4)
Mae Taeng	Cho Lae	41	18 (43.9)	45	9 (20.0)	86	27 (31.4)
San Sai	Nang Yaeng	35	1 (2.9)	49	2 (4.1)	84	3 (3.6)

Discussion

This present study was able to demonstrate that there is still a need for interventional programs regarding *Opisthorchis viverrini* infections in MIF endemic areas. Probable targets to minimize transmission are the standard of personal and public hygiene as well as education on eating and cooking behaviours. Parasitic infections were found in 36 cases (11.0%). The majority of detected parasites (17 cases, 5.2%) were *Opisthorchis viverrini*. Other intestinal parasites included *Trichuris trichiura*, hook-worm, and minute intestional fluke. The highest prevalence (18.2%) was found in villagers aged 41-50 years.

Differences in the prevalence of infection presumably reflect variations in environmental conditions as well as social behavior. The crude overall prevalence of infection in our study population was lower than the overall prevalence rate of 15.7% for Northeast Thailand observed in 2001. The variability between different geographic regions, but also seasonal and cultural variations might serve as an explanation for this discrepancy. Further studies are required to "up-date" the health status and health care needs in certain rural areas of Thailand. Additional studies on the efficacy of interventional programs that address health behaviour are recommended. Perhaps, this type of approach might offer a comprehensive strategy for the helminth dilemma.

"Population health" is a public health approach that targets individuals at high risk and might offer a cost effective way to allocate limited funds. The present study was able to demonstrate that there is still a need for further control programs regarding *O. viverrini* infections in rural areas. *Opisthorchis viverrini*, a well-described etiologic factor for cholangiosarcoma, was found in more than 5% of all subjects. Overall, males were more often infected than females. Similar findings were observed in other endemic communities. Within the northeast itself, there is a large variability at the provincial, district, and village level with a prevalence of infection slightly higher among males [5, 8-11]. However, when compared with numbers from 1981, where 34.6% of all observed people in the Northeast were infected with *O. viverrini*, the success of the implemented liver fluke control program is demonstrated impressively [5]. Nevertheless, data derived from our study demonstrated that there are still groups predisposed to higher infection rates. We were able to show that lower education is associated with higher prevalence rates, which might reflect the unevenness of access to information. Therefore, educating eating and cooking behaviors remains a crucial tool in the control of liver fluke infections. The educational programs should target individuals and communities at risk, and further sociological studies are recommended to elucidate risk behaviors in the transmission of opisthorchiasis and other intestinal parasites.

Further studies are required to "update" the current health status of populations in specific endemic areas of Thailand, and to investigate the impact of socioeconomic background on their health behavior. Perhaps, this type of approach might offer a comprehensive and cost-effective strategy for the helminth dilemma.

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