

AN EPIDEMIOLOGICAL STUDY ON *OPISTHORCHIS VIVERRINI* INFECTION IN LAO VILLAGES

Jun Kobayashi^{1,2}, Bouakham Vannachone², Yoshiya Sato¹, Khempavanh Manivong³,
Simone Nambanya² and Souliya Inthakone²

¹Department of Parasitology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan; ²Center of Malariology, Parasitology and Entomology (CMPE), Ministry of Health, Vientiane, Lao PDR; ³Malaria Station, Khammouane Provincial Health Office, Khammouane, Lao PDR

Abstract. The prevalence of liver fluke, *Opisthorchis viverrini*, infection in rural and urban communities was studied in Khammouane Province in Lao PDR. The infection was the commonest among the villagers examined, showing the positive rates of 52.9% and 55.0% in two rural communities and 60.7% in an urban community, respectively. The infection rate reached up to 20% or more within 4 years after birth and increased with age to a plateau in the age group over 20 years. The highest prevalence rate was over 80% in the age group of 35-54 years. The age-related patterns of infection in males and females were almost the same, although there were some sex-related differences by village and by age group. Infection with *Opisthorchis viverrini* appears to be a serious public health problem strongly associated with the frequent eating habit of raw fish in low land Laos.

INTRODUCTION

The liver fluke, *Opisthorchis viverrini*, is commonest human intestinal parasite in northeast Thailand (Preuksaraj, 1984; Upatham *et al.*, 1985; Brockelmen *et al.*, 1987) and low land Laos (Giboda *et al.*, 1991; Pholsena *et al.*, 1991; Scholtz *et al.*, 1992; Kobayashi *et al.*, 1996). The infection is known to be acquired by ingestion of raw fresh water fish. In both areas, residents have kept same habit frequently eating raw fresh water fish. A number of researchers have reported the epidemiological features of this infection in northeast Thailand. The prevalence among rural dwellers is higher than among urban dwellers (Kurathong *et al.*, 1987). The prevalence and intensity of the infection increased with age, reaching a plateau in young adults (Haswell-Elkins *et al.*, 1991). Hepatobiliary disease is produced in human during the infection. It is serious public health problem by virtue of its association with cholangiocarcinoma which causes a considerable morbidity and mortality in the area (Vatanasapt *et al.*, 1990; Parkin *et al.*, 1991; Haswell-Elkins *et al.*, 1992; Sithithaworm *et al.*, 1994). The average age of cholangiocarcinoma patients seeking treatment in endemic areas is 2-

3 decades earlier compared to non-endemic areas, and it was reported that the hepatobiliary disease frequently accompanies heavy infection. It was suggested that the priority for community-based *Opisthorchis* control programs should be to maximize treatment and health promotion efforts toward heavily infected dwellers (Elkins *et al.*, 1990; Haswell-Elkins *et al.*, 1991).

In Lao PDR, an almost 100% rate among adults was reported in communities in Vientiane Province (Pholsena *et al.*, 1991). The intensity of this infection was also studied in one electric power station camp and one village in Vientiane Province (Giboda *et al.*, 1991). More recently, the authors reported that 38.6% of children was infected in rural communities in Khammouane Province located 350 km southeast from Vientiane (Kobayashi *et al.*, 1996). However, the detailed epidemiological features in the communities has not been fully delineated, thus, in the present study, we have studied the prevalence and intensity of infection in all age groups in two rural villages and one urban village.

MATERIALS AND METHODS

Study area and subjects

Three villages were selected as survey area in Khammouane Province where anti-parasite control programs had been operated as primary health care activities under Lao PDR-Japan international technical cooperation. Two out of the three villages, Sisomsouen

Correspondence: Jun Kobayashi, Department of Parasitology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan.
Tel: +81-98-895-1129; Fax: +81-98-895-1409; E-mail: jicaimpe@laotel.com

Village and Phavang Village, are located about 55 km north from Thakhek City, capital city of the province. Both villages belong to the rural area are surrounded by paddy fields. The socio-economic and sanitary situations, however, are somewhat different between the two villages. Approximately 20% of households have latrines around their own houses in Sisomsouen Village but latrines are not yet present near any house in Phavang village. The dwellers in Sisomsouen village can get income from not only farming and hunting, but also from commerce and manual industry.

The remaining village surveyed was Thakhek Neua village located inside Thakhek City. The village is belonging to an urban area with relatively good sanitary conditions; almost all households have a latrine in or around their houses. The ethnic group of the villagers in the study areas was Lao lum, the majority people in Laos, who have the habit of eating raw fish frequently.

The total populations in the villages were approximately 400 in Sisomsouen village, 350 in Phavang village and 500 in the target area of Thakhek Neua village, respectively. A total of

671 villagers, accounting for 44% to 73% of the total, received stool examinations. The age and sex distributions of subjects examined are shown in Table 1. More than half of the villagers examined were children and teenagers under 20 years old. Female subjects comprised 54.1% of the villagers examined.

Stool examination

A single stool sample was collected from each villager after the delivery of stool containers and instruction for stool collection in December 1996. The stool samples were examined by the Kato-Katz thick smear method within 2 days after collection. The size of a punched hole in a vinyl template used in the present survey was 6 mm in diameter and 1 mm deep to hold approximately 33 mg of feces. The preparation was allowed to stand about 30 minutes at room temperature and was then examined microscopically. The intensity of infection was calculated by estimating the total number of eggs in the 33 mg feces preparation and expressed as eggs per gram feces (EPG).

Statistics

Statistical analysis was performed using com-

Table 1
The results of stool examination for *Opisthorchis viverrini* infection among the villagers examined.

Age group (years old)	Male		Female		Total	
	No. exam	No. positive (%)	No. exam	No. positive (%)	No. exam	No. positive (%)
Sisomsouen village						
0-4	12	1 (8.3)	14	4 (28.6)	26	5 (19.2)
5-9	14	5 (35.7)	16	5 (31.3)	30	10 (33.3)
10-14	15	9 (60.0)	12	6 (50.0)	27	15 (55.6)
15-24	5	5 (100)	14	8 (57.1)	19	13 (68.4)
25-	51	39 (76.5)	36	18 (50.0)	87	57 (65.5)
Total	97	59 (60.8)	92	41 (44.6)	189	100 (52.9)
Phavang village						
0-4	15	2 (13.3)	20	6 (30.0)	35	8 (22.9)
5-9	25	10 (40.0)	23	7 (30.4)	48	17 (35.4)
10-14	26	14 (53.8)	14	5 (35.7)	40	19 (47.5)
15-24	10	4 (40.0)	8	5 (62.5)	18	9 (50.0)
25-	56	44 (78.6)	61	45 (73.8)	117	89 (76.1)
Total	132	74 (56.1)	126	68 (54.0)	258	142 (55.0)
Thakhek Neua village						
0-4	21	3 (14.3)	22	9 (40.9)	43	12 (27.9)
5-9	26	13 (50.0)	31	18 (58.1)	57	31 (54.4)
10-14	17	11 (64.7)	26	17 (65.4)	43	28 (65.1)
15-24	1	1 (100.0)	16	12 (75.0)	17	13 (76.5)
25-	12	10 (83.3)	52	42 (80.8)	64	52 (81.3)
Total	77	38 (49.4)	147	98 (66.7)	224	136 (60.7)

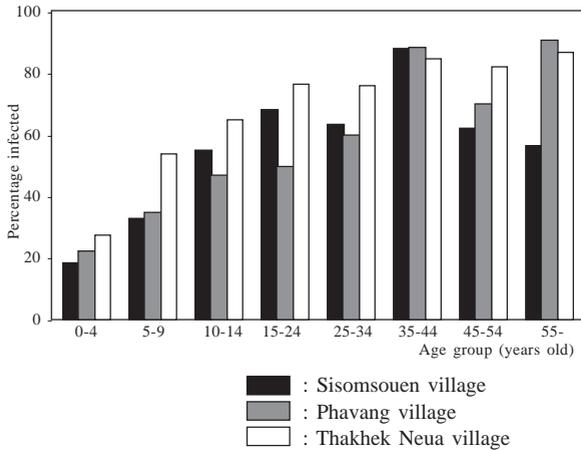


Fig 1-The age-related prevalence of *Opisthorchis* infection among three villages.

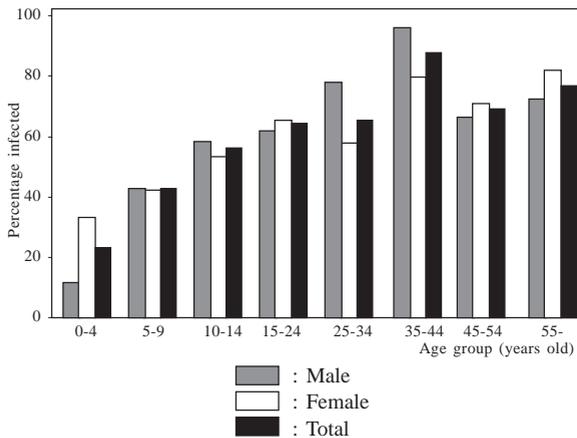


Fig 2-The age-related prevalence of *Opisthorchis* infection in male and female groups.

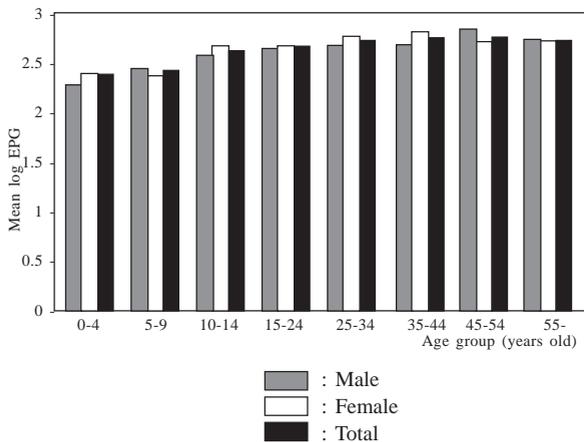


Fig 3-The age-related intensity of *Opisthorchis* infection estimated by EPG in male and female groups.

puter statistical software (SPSS). The probability (p-value) less than 0.05 was considered to be statistically significant.

RESULTS

Infection with any helminth parasite was determined to be as high as 73-86% among the villagers examined. Eight species of intestinal helminths were demonstrated; the most common parasite was *Opisthorchis viverrini*. The prevalence of *O. viverrini* infection based on egg detection rate was estimated to be 52.9% in Sisomsouen village, 55.0% in Phavang village and 60.7% in Thakhek Neua village, respectively (Table 1). Although the difference was not statistically significant, the positive rate was higher in urban village, Thakhek Neua village, than the other two rural villages. When the positive rates were compared by each age group (Table 1), about 20% or greater positive rates were observed in infants under 4 years old and reached 47-65% in the following 10 years. The positive rates were 50% or more in adults over 20 years old. When age-related prevalence was further compared (Fig 1), the infection rates showed similar linear increase with age to plateaus in the age group from 35 to 44 years old in all villages. The sex-related prevalence is also presented in Table 1. The positive rate in male villagers was higher than in females in Sisomsouen village but the reverse was true in Thakhek Neua village. The rates were almost the same in males and females in Phavang village. In Fig 2, the positive rates are further compared between male and female villagers by age group. The positive rate was higher in females in infants under 4 years old and in males in the age group from 25 to 44 years old. Fig 3 represents the intensity of the infection estimated by logarithmic EPG in each age and sex group. The intensity of the infection did not differ between age groups and sex groups, showing that individuals acquire heavy infection at an early age and maintain it for most of their lifetime in both urban and rural areas.

Among the villagers positive for *O. viverrini*, 101 villagers received anthelmintic treatment with praziquantel (single dose of 40 mg/kg). Out of the 83 villagers who received follow up stool examination, only 8 (9.6%) villagers revealed positive results 1 month after the treatment. The positive rate, however, increased to 15.4% (12/78) at 3 months and 30.2% (19/63) at 6 months after the treatment. As the result, 12 (16.0%) out of 75 villagers

followed-up after successful treatment were confirmed to acquire the re-infection within a half year after treatment.

DISCUSSION

The results presented here demonstrated that *Opisthorchis* infection is highly prevalent among the villagers examined in Lao PDR, as well as in neighboring northeastern Thailand. As an interesting finding in Laos, the positive rate was higher in an urban village, where the prevalence of other soil-transmitted helminths was significantly lower, than in the two rural villages. In neighboring northeastern Thailand, it has been reported that positive rate and intensity were consistently low (*ie* less than 10% positive rate) in children and it increased steady as increase of age of the subjects (Brockelman *et al*, 1987; Kurathong *et al*, 1987; Haswell-Elkins *et al*, 1991). In contrast, in the villages studied here, a positive rate as high as 24% was already attained in the same age group and it reached more than 50% within 15 years after birth. The intensity of the infection estimated by EPG was also significantly higher in young villagers as compared with those in Thailand (Kurathong *et al*, 1987; Haswell-Elkins *et al*, 1991). These results indicate that the initial infection with *O. viverrini* occurs in early childhood in Lao PDR and remains high for a long period in adulthood. The eating habits in the present villages may be a major reason why so many infants are infected with the parasite as compared with Thailand. Weaning is sometimes started with the same food the mother eats, for babies under 1 year old in Lao communities. This eating habit may normally be more frequent in rural areas than urban areas (Changbumrung *et al*, 1989). The prevalence rate, however, was rather than higher in the urban area than in the rural areas in the present study, although children under 10 years old, whose prevalence rate was generally low as compared to the adults, occupied 64% of the subjects in the present urban village. An explanation that could be given is that the habit eating raw fish is frequent among urban villagers as their normal meal, as well as particular social and traditional events of Lao tradition.

In the present study, it was shown that infection with this parasite begins at very early age and remains high thereafter. High incidence of *O. viverrini* infection in the children may be a risk factor in the production of serious liver disease after a long-

standing infection in adulthood. *O. viverrini* infection is known to lead to serious health problems in endemic areas because of its serious pathogenicity (Elkins *et al*, 1990; Mairiang *et al*, 1992). In endemic areas, the infection is associated with clinical symptoms such as abdominal pain, weakness, flatulence, composition of gallstone, hepatomegaly, in relation to the intensity of the infection (Upatham *et al*, 1982; 1984; Riganti *et al*, 1988; Hou *et al*, 1989; Shirai *et al*, 1992). Additionally, there is strong epidemiological and experimental evidence supporting an important etiological role of the parasitic infection in the development of cholangiocarcinoma in humans (Sonakul *et al*, 1978; Flavell and Lucas, 1982; Vatanasapt *et al*, 1990; Parkin *et al*, 1991; Haswell-Elkins *et al*, 1992; Sithithaworn *et al*, 1994). Although there have been no investigation from such a point of view in Lao PDR, the authors have also recognized abnormal findings in the hepatobiliary region frequently among the opisthorchiasis patients in the study areas by ultrasonographic examination of liver (unpublished data). Although further epidemiological studies are necessary to clarify the relationship between the infection and hepatobiliary carcinogenesis in this country, the mothers and early age children should be target group for treatment and health education in community based *Opisthorchis* control to avoid the development of cholangiocarcinoma, because high prevalence and high intensity of infection is present among early age children in Lao communities.

Finally, 16.0% of the patients who received successful treatment with praziquantel acquired re-infection within 6 months after the treatment. The infection may occur through frequent consumption of favorite dishes, Koipa, Larrppa and so on, prepared from uncooked freshwater fish, which contains viable metacercariae. However, there have been few studies in Laos on the infection rate of metacercariae in freshwater fish as an important infection source of the humans. From the public health standpoint, a widespread campaign to reduce transmission of the parasite using a combination of education, improved eating habits and drug therapy should be made to eradicate or control the highly prevalent parasitic disease.

ACKNOWLEDGEMENTS

This study was performed under Lao/Japan medical cooperation project on primary health care

supported by Japan International Cooperation Agency (JICA). The authors would like to thank all staff member in Ministry of Health and CMPE, Lao PDR and JICA Lao PDR Office, for their helpful supports and advice in this study. We would also like to thank staff members in Khammouane Provincial Health Office, Hinboon District Health Office and Thakhek District Health Office for their sample collection, examination and implementation of the surveys.

REFERENCES

- Brockelman WY, Upatham ES, Hirunraks A, *et al.* Measurement of incidence of the human liver fluke, *Opisthorchis viverrini*, in northeast Thailand. *Trans R Soc Trop Med Hyg* 1987; 81: 327-35.
- Changbumrung S, Tungtrongchitr R, Hongtong K, *et al.* Food patterns and habits of people in an endemic area for endemic area for liver fluke infection. *J Nutrit Assoc Thai* 1989; 23: 133-46.
- Elkins DB, Haswell-Elkins MR, Mairiang E, *et al.* A high frequency of hepatobiliary disease and suspected cholangiocarcinoma associated with heavy *Opisthorchis viverrini* infection in a small community in northeast Thailand. *Trans R Soc Trop Med Hyg* 1990; 84: 715-9.
- Flavell DJ, Lucas SB. Potentiation by the human liver fluke, *Opisthorchis viverrini*, of the carcinogenic action of N-nitrosodimethylamine upon the biliary epithelium of the hamster. *Br J Cancer* 1982; 46: 985-9.
- Giboda M, Ditrich O, Scholz T, *et al.* Current status of food-borne parasitic zoonoses in Laos. *Southeast Asian J Trop Med Public Health* 1982; 46 (suppl): 56-61.
- Giboda M, Ditrich O, Scholz T, *et al.* Human *Opisthorchis* and *Haplorchis* infections in Laos. *Trans R Soc Trop Med Hyg* 1991; 85: 538-40.
- Haswell-Elkins MR, Elkins DB, Sithithaworn P, *et al.* Distribution patterns of *Opisthorchis viverrini* within a human community. *Parasitology* 1991; 103: 97-101.
- Hou MF, Ker CG, Sheen PC, *et al.* The ultrasound survey of gallstone diseases of patients infected with *Clonorchis sinensis* in southern Taiwan. *J Trop Med Hyg* 1989; 92: 108-11.
- Kobayashi J, Vannachone B, Xeuatvongsa A, *et al.* Prevalence of intestinal parasitic infection among children in two villages in Lao PDR. *Southeast Asian J Trop Med Public Health* 1996; 27: 562-5.
- Kurathong S, Lerdverasirikul P, Wongpaitoon V, *et al.* *Opisthorchis viverrini* infection in rural and urban communities in northeast Thailand. *Trans R Soc Trop Med Hyg* 1987; 81: 411-4.
- Mairiang E, Elkins DB, Mairiang P, *et al.* Relationship between intensity of *Opisthorchis viverrini* infection and hepatobiliary disease detected by ultrasonography. *J Gastroenterol Hepatol* 1992; 7: 17-21.
- Parkin DM, Srivatanakul P, Khlat M, *et al.* Liver cancer in Thailand. I. A case-control study of cholangiocarcinoma. *Int J Cancer* 1991; 48: 323-8.
- Pholsena K, Sayaseng B, Hongvanthong B, *et al.* The prevalence of helminth infection in Ban Nanin, Laos. *Southeast Asian J Trop Med Public Health* 1991; 22: 137-8.
- Preuksaraj S. Public health aspects of opisthorchiasis in Thailand. *Drug Res* 1984; 34: 1119-20.
- Riganti M, Pungpak S, Sachakul V, *et al.* *Opisthorchis viverrini* eggs and adult flukes as nidus and composition of gallstones. *Southeast Asian J Trop Med Public Health* 1988; 19: 633-6.
- Scholz T, Ditrich O, Gutvirth J, *et al.* The prevalence of helminth infection in Ban Nanin, Laos: additional data. *Southeast Asian J Trop Med Public Health* 1992; 23: 802-3.
- Shirai T, Pairojkul C, Ogawa K, *et al.* Histomorphological characteristics of cholangiocellular carcinomas in northeast Thailand, where a region infection with the liver fluke, *Opisthorchis viverrini* is endemic. *Acta Pathol Japonica* 1992; 42: 734-9.
- Sithithaworn P, Haswell-Elkins MR, Mairiang P, *et al.* Parasite-associated morbidity: Liver fluke infection and bile duct cancer in northeast Thailand. *Int J Parasitol* 1994; 24: 833-43.
- Sonakul D, Koopirochana C, Chinda K, *et al.* Hepatic carcinoma with opisthorchiasis. *Southeast Asian J Trop Med Public Health* 1978; 9: 215-9.
- Upatham ES, Viyanant V, Kurathong S, *et al.* Morbidity in relation to intensity of infection in *Opisthorchis viverrini*: study of a community in Khon Kaen, Thailand. *Am J Trop Med* 1982; 31: 1156-63.
- Upatham ES, Viyanant V, Kurathong S, *et al.* Relationship between prevalence and intensity of *Opisthorchis viverrini* infection, and clinical symptom and signs in a rural community in northeast Thailand. *Bull WHO* 1984; 62: 451-61.
- Upatham ES, Brockelman WY, Viyanant V, *et al.* Incidence of endemic *Opisthorchis viverrini* infection in a village in northeast Thailand. *Am J Trop Med Hyg* 1985; 34: 903-6.
- Vatanasapt V, Tangvoraphonkchai V, Titapant V, *et al.* A high incidence of liver cancer in Khon Kaen Province, Thailand. *Southeast Asian J Trop Med Public Health* 1990; 21: 489-94.