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A REVIEW OF EXPERIMENTAL AND FIELD RESEARCH ON THE HUMAN LIVER FLUKE, *OPISTHORCHIS VIVERRINI*

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ABSTRACT

Opisthorchiasis is caused by a liver fluke, Opisthorchis viverrini, *which afflicts approximately seven million inhabitants in the northeast and north of Thailand. The parasite utilizes, respectively, freshwater snails and cyprinoid fish as its first and second intermediate hosts. Man is the accidental definitive host who acquires infection through the consumption of improperly cooked cyprinoid fish. To assist beginning researchers, information concerning experimental and field research on various aspects of* O. viverrini, *especially the epidemiology and transmission dynamics, is compiled and presented.*

INTRODUCTION

Human opisthorchiasis in Thailand and neighbouring countries is caused by a liver fluke, *Opisthorchis viverrini.* The parasite is classified in Phylum Platyhelminthes, Class Trematoda, Order Prosostomata and Family Opisthorchiidae.

Opisthorchis viverrini was first described in the postmortem examination of two prisoners from a jail in Chiengmai, northern Thailand, in 1911 by Leiper,[1] who obtained specimens from Kerr. Five years later, Kerr[2] reported that 17 per cent of 230 adult male prisoners examined in a prison in Chiengmai were infected with *O. felineus.* A decade later, Prommas[3] identified the worms found at an autopsy of a 17-year-old Thai male residing in Roiet, northeastern Thailand, as *O. felineus.* At the same period of time, Bedier and Chesneau[4] reported finding

O. viverrini infection among the Laotian people in Vientiane (15%) and Takhek (23%). Further basic knowledge on liver fluke infections in Thailand was contributed by Sadun,[5] Harinasuta and Vajrasthira,[6,7] and Wykoff *et al.*[8] Sadun[5] commented that the liver fluke infection in Thailand was caused by *O. viverrini* (not by *O. felineus*), and this was confirmed later by Wykoff *et al.* [8]

MORPHOLOGY AND LIFE CYCLE

Opisthorchis viverrini is a liver fluke of cats and dogs, and man is the accidental definitive host. The parasite utilizes freshwater snails and various species of cyprinoid fish, respectively, as its first and second intermediate hosts. Man acquires infection by consuming improperly cooked fish (Fig. 1).

The Adult

The adult worms of *O. viverrini* are flat, leaf-shaped, transparent and hermaphroditic flukes. In man, the size of mature worms ranges from 5.4 to 10.2 mm (mean 7.0 mm) in length and 0.8 to 1.9 mm (mean 1.5 mm) in width. In cats and dogs the size is smaller, from 3.1 to 7.4 mm (mean 4.6 mm) in length and 0.7 to 2.9 mm (mean 2.0 mm) in width.[5] The adult flukes from human and animal sources are alike;[5] they live in the bile and pancreatic ducts, and occasionally in the gallbladder.[9,10] The living parasite is reddish yellow in colour when newly removed from the bile duct (Fig. 2).[9]

The worms in animals take approximately one month to mature. The eggs travel through the bile ducts of man or animals, enter the lumen of the intestines and pass out of the definitive host with the faeces. A mature worm will lay approximately 3,160 eggs (range: 2,000 to 4,200) per day in humans, and 2,830 eggs (range: 358 to 3,509) and 2,353 eggs (range: 1,870 to 4,542) per day in cats and rabbits, respectively.[11]

The Egg

The egg of *O. viverrini* is yellowish brown in colour. It is usually oval in shape, with a cup-like operculum resting on its shoulders with a frequently present tubercle-like appendage in the middle of the abopercular end.[5] The size of the egg varies from 19 to 29 microns (mean 26.7 microns) in length and 12 to 17 microns (mean 15 microns) in width, and the eggs from cats and humans are alike morphologically.[5] The eggs are excreted with the faeces, and when they are laid the ciliated miracidia are fully developed (Fig. 3).[9,10]

The Larval Stages

The snail intermediate host

Hatching of the *O. viverrini* eggs does not occur in water; they have to be first ingested by appropriate freshwater snails. *Bithynia (Digoniostoma) funiculata* is the first intermediate host of *O. viverrini* in northern Thailand, whereas *Bithynia (Digoniostoma) siamensis goniomphalos* and *Bithynia (Digoniostoma) siamensis siamensis* occur in northeastern and central Thailand, respectively.[8,12] The snails are commonly found in standing-water habitats, such as reservoirs, ponds, rice fields, etc., and are classified in Phylum Mollusca, Class Gastropoda, Order Mesogastropoda, and Family Bithyniidae (Fig. 4).[12]

When the *O. viverrini* eggs are ingested by the snail, they hatch and become miracidia in the digestive tract of the snail. The miracidium is a top-shaped organism, 32 microns in length and 17 microns in width. The miracidia then penetrate into the lymph spaces where they become sporocysts. The immature sporocyst migrates along the periintestinal lymph sinuses, elongates, becomes inactive, and produces a large number of developing rediae. The mature sporocyst is thin-walled, and its average size is 1.1 by 0.65 mm. The developing rediae exhibit distinct contractions which help them in breaking out from the thin-walled sporocyst. The active rediae migrate to the interhepatic lymph sinuses, where they reach maturity. The size of rediae ranges from 0.18 to 1.1 mm in length and 0.08 to 0.28 mm in width (mean: 0.54 by 0.12 mm). The redia possesses a distinct pharynx, thick integument, and within it are as many as 15 developing cercariae. The total length of cercariae varies from 490 to 565 microns (mean: 532). The size of the body ranges from 140 to 183 microns (mean: 154) in length and from 61 to 96 microns (mean: 75) in width, while that of the tail is 26 microns. At maturity, cercariae break out of the redia into the interhepatic lymph spaces, penetrate the tunica propria of the snail and escape into the water (Fig. 5).[8,13] When at rest, they assume a characteristic pipe form, and are both geo- and photo-tropic.[8] The cercaria is of the pleurolophocercous type.[14] The development of the *O. viverrini* intramolluscan stages takes 8 to 10 weeks.[15]

The fish intermediate host

The cercaria is a free-swimming stage and can exist in the water for 24 to 48 hours, but when inactive it sinks to the bottom. Unless it encounters an appropriate fish within this period, it perishes.

The cercaria penetrates under the scales of cyprinoid fishes, then loses its tail and becomes an oval cyst called metacercaria. Encystment takes place mainly in the head portion and the body muscle, and incidentally in the fins, skin and scales.[16-18] The metacercaria is contained in a double-walled cyst. The walls of the

cyst are thin, the outer being 3 to 8 microns thick and the inner so thin that it can be recognized only after the parasite has escaped. The average size of the cyst is 202 microns in length and 168 microns in width. The metacercarial body in the cyst frequently appears to be C-shaped. The characteristic excretory corpuscles in the bladder, the brownish-yellow pigment, and the oral and ventral suckers are usually evident. The body of the excysted metacercaria averages 558 microns in length and 145 microns in width (Fig. 6).[19]

The cyprinoid fishes that serve as the second intermediate host of *O. viverrini* belong to the Family Cyprinidae, such as *Cyclocheilichthys apagon, C. armatus, C. siaja, Puntius leiacanthus, P. orphoides, P. viehoever, P. partipentazona, Hampala dispa,* etc. (Fig. 7).[8,16,17,20] The metacercarial cysts can resist environmental factors well. They remain viable and infective in the refrigerator at 4° to 6°C for 16 days after the death of the fish, but die after 32 days at such temperatures[21] and are not infective in 6.8% salinity after 48 hours.[22]

After the metacercariae are ingested by a definitive host, they excyst in the duodenum by the action of gastric and duodenal juices. Then, the liberated metacercariae migrate through the ampulla of Vater to the bile ducts, pancreatic duct, and gallbladder, where they attach themselves to the epithelial lining and mature within 3 to 4 weeks.[9-12]

The entire life cycle of *O. viverrini* requires from 4 to 4.5 months. The worms have a life span of 25 to 30 years.[9,10]

EPIDEMIOLOGY AND TRANSMISSION DYNAMICS

Opisthorchiasis is endemic among human populations in the northeast and north of Thailand, where the most common raw fish "koi-pla" is frequently consumed (Fig. 8).[5,8,18,23] In the central region where no indigenous human infection is ever encountered, cats and dogs are found naturally infected with the human liver flukes. In the southern region, no infection is encountered.[5] It has been estimated by various investigators that the fluke afflicts up to 7 million people in Thailand.[5,6,8,23]

The Human Host

Prevalence of infection

The main endemic areas are located widely in northeastern Thailand and in some provinces in northern Thailand.[5,6] Prevalence rates (measured as the number of cases occurring during a specified period of time) in selected groups of inhabitants in the northeast and the north were, respectively, 29.8% (range: 3.0 to 87.7) and 10.3% (range: 0.5 to 20.5).[24] In the northeast, the *O. viverrini* infections can be divided into areas of high prevalence and relatively low prevalence.

The areas of high prevalence (such as Kalasin, Mahasarakarm, Udornthanee, Sakolnakorn, Nongkai, etc.) are concentrated along the valley of the Mekong River, which receive the flow from the Mekong River and its tributaries, and in which numerous marshes, swamps and lakes are situated (Fig. 9). The areas of relatively low prevalence (such as Nakornrajsima, Burirum, Surin, Srisaket, etc.) are situated close to central Thailand and far removed from the Mekong River.[5] Moreover, the prevalence among inhabitants living along the shoreline of lakes was higher than that among those living at a distane from them.[5] It is also documented that prevalence was higher among inhabitants residing at the periphery of an old man-made lake than among those residing near a new man-made lake.[25,26]

Intensity of infection

Recent cross-sectional studies of two villages (Chonnabot and Nong Ranya, Khon Kaen province) in a hyperendemic area showed that infection with *O. viverrini* began at a very early age; prevalence of infection rose rapidly with age up to adulthood and remained relatively high thereafter, the relationship of prevalence to age being a function of "koi-pla" consumption. The intensity of infection (faecal egg output) in both males and females rose steadily in early life, reached highest in the 40-49-year age group and remained relatively constant through older ages. In all age groups, the prevalence and intensity of infection in both men and women were similar.[27-29] It has also been shown that the prevalence and intensity of infection were greater among rural dwellers than their urban counterparts, an observation strongly associated with the habit and frequency of eating "koi-pla".[30]

Incidence of infection

At Chonnabot village, the incidence (measured as the proportion of persons whose stools become positive within one year) of *O. viverrini* infection was analyzed over a two-year period with particular reference to age, sex and changes in the availability of infective stages. The incidence was found to be higher in males than in females, especially in children under five years of age. It was at least 47% overall in the first year of the study, but declined to below 20% per year in the second. This is attributed to the drying up of a local water reservoir and the decline in the availability of infective stages in fish. The fluctuation of incidence is probably due to the large variations in rainfall from year to year. The rate of reversion from positive to negative varied from 2% to 6% per year.[31,32]

Rate of re-infection

At Chonnabot village, patterns of re-infection by *O. viverrini* were studied over a period of one year after chemotherapy (using Praziquantel). It was found that 88.4% of those examined after 2 weeks had negative stools. However, within one year of treatment, 88.7% of the cured subjects who were re-examined had become re-infected, and 51.5% had infections of at least moderate intensity (1 egg

per milligramme of faeces), as compared to 72% of the same group before treatment. Also, those with high pre-treatment intensities of infection tended to have more intense re-infections, indicating that some people are predisposed to heavy infections. The rate of re-infection of those with high pre-treatment intensity of infection was about twice that of those who were negative or who only had light infection before treatment.[38] High re-infection rates were also found in a recent study of control of opisthorchiasis at a large irrigation reservoir in the same province in northeastern Thailand.[34] The investigator commented that chemotherapy would have to be applied several times a year in order to control opisthorchiasis, and suggested that it might be most cost-effective to preferentially treat heavily infected individuals.[33]

Morbidity in relation to intensity of infection

In a recent study at Chonnabot and Nang Ranya villages, it was found that the percentage of the population with symptoms and signs attributable to opisthorchiasis were in the following order: 5 to 10% for weakness or malaise, flatulence or dyspepsia and abdominal pain, and 5% for hepatomegaly, which symptoms were related to the intensity of infection. The ability to work and signs of nausea, vomitting, diarrhoea, and splenomegaly, however, were unrelated to the intensity of opisthorchiasis.[27,28] The quoted proportions are high enough to indicate that opisthorchiasis is a disease of public health significance in heavily infected villages, and not one that produces medical complications in only rare cases.

The intermediate hosts

There have been two field studies on the transmission dynamics of *O. viverrini* in, and population changes of, *B.s. siamensis* and *B.s. goniomphalos* in central and northeastern Thailand, respectively, over a period of one to three years.[35,36] In canals in Bangkok where snail habitats were deep during the high rainfall period, snail populations recovered at the water surface were higher than those recovered on the mud bottom; during the low rainfall period when snail habitats were shallow, snails were found copiously both at the water surface and on the mud bottom. Snails infected with *O. viverrini* could be found almost all year round, with an overall infection rate of 1.6%.[35] In a shallow water reservoir in Khon Kaen province, where the habitat experienced a 17-month drought during the 3-year study period, followed by a surging flood, snails suffered over 90% mortality during drying out of the reservoir, but died at an average rate of only 5% per month during aestivation. When water returned they migrated to new edge habitats and reproduced. Peaks in reproduction occurred following spring rains and after the fall monsoon flooding subsided. Normally, two generations are produced per year in seasonal habitats. The prevalence of *O. viverrini* infection averaged 0.11% (Fig. 10).[36]

Surveys of *O. viverrini* metacercariae in cyprinoid fish in lakes, ponds, streams and markets of 7 northeastern provinces were conducted. Out of 1,907 fish examined, 35.7% (range: 19.7 to 60.4) were found to be infected, with a mean of 8.7 metacercariae per fish (range: 3.5 to 10.8).[7,20] The metacercariae were found in all parts of the cyprinoid fish body,[7,16,17,20] with the highest number of distribution in the head region[17] and in the body muscle.[16] Monthly variations in intensities of *O. viverrini* infection in *C. apagon* and *P. leiacanthus* in Nong Kong Kaew, Khon Kaen province were investigated for over one year.[16] The overall infection rates were 97.1% and 93.7%, respectively, for *C. apagon* and *P. leiacanthus.* Both species of fish were found infected throughout the year, with the period of high intensity of infection being between September and February, and with the number of metacercariae per fish ranging from 51.8 to 88.5 (Fig. 11).[16]

PATHOLOGY

The pathologic changes produced by *O. viverrini* are localized in the distal biliary passages, particularly those of the left lobe of the liver. The injury to the host depends upon the number of flukes; a few parasites cause little damage, but large numbers produce serious and progressive hepatic disease.[13] The liver is grossly normal in light infections, but in heavy infections the liver may become enlarged. Furthermore, there are localized dilatation of the slightly thickened bile ducts, proliferation of biliary ductal epithelium and some atrophy of the parenchymal cells.[9,37] Adenomatous or ductular hyperplasia, periductal fibrosis and eosinophilic infiltration are evident.[10] The dilatation of the bile ducts may be of mulberry type, or saccular type, or cystic formation.[38,39]

There are some evidences indicating that obstruction of the bile ducts (from the flukes), irritation to the biliary ductal epithelium (from ventral suckers and the flukes) and reaction to toxins (secreted by the flukes or eggs) or immunopathologic mechanisms (antigens released from the flukes or eggs) may be important in the pathogenesis of opisthorchiasis.[39,40] Moreover, secondary bacterial infection, malnutrition, the presence of large numbers of flukes in the bile ducts and the duration of infection may also be important factors in causing pathogenesis.[39-41]

In patients with heavy and chronic infections of *O. viverrini,* associated diseases may occur, such as obstructive jaundice,[9,37-39] ascending cholangitis or oriental cholangiohepatitis,[42] liver cyst,[43,45] cholelithiasis,[37,46-48] hydrop of gallbladder,[45] carcinoma of cystic duct,[49] biliarobronchial fistula,[50] biliary cirrhosis[9] and cholangiocarcinoma.[51-58] In hamsters infected with *O. viverrini,* cholangiocarcinoma can be produced by the administration of dimethylnitrosamine.[51,52] In a recent case-controlled study, it has been shown that patients infected with *O. viverrini* render a higher rate of cholangiocarcinoma than do patients without such infection.[48]

It has been shown that although patients infected with *O. viverrini* have low absorption of vitamin B_{12}, they have normal serum vitamin B_{12} level in them, probably because most Thai people consume fish sauce and fermented fish which are very rich in vitamin B_{12}.[59-61] Also, there are no pathologic effects of the oral contraception and the injectable contraceptive depot medroxyprogesterone acetate in Thai women infected with opisthorchiasis.[62-64]

PROTECTIVE IMMUNITY

Immunologic studies on *O. viverrini* are scarce. The literature concerning the immunology of *O. viverrini* infection deals mainly with immunodiagnosis and serology, such as immunoelectrophoresis,[65,66] immunodiffusion,[66] enzyme immunoassay,[67] passive haemagglutination,[68] radio immunoprecipitation and autoradiography,[69] indirect immunofluorescence,[70] and enzyme-linked immunosorbent assay.[71,72]

Information regarding the protective immunity in *O. viverrini*-infected animals has become available during the last few years. It has been shown by some investigators that protective immunity does not occur in hamsters receiving serum or spleen cells from *O. viverrini*-infected donors,[73] or prior infection of hamsters with *O. viverrini* fail to induce significant protection against re-infection.[74] The absence of protective immunity may be attributed either to the failure of the host to elicit an effective immune response against the appropriate parasite antigen (s) or to the invasion of parasites on the host immune defenses.[74]

CONTROL

Transmission of *O. viverrini* infection requires consumption of improperly-cooked cyprinoid fish, the second intermediate hosts, in an area where sanitation is at a low level and there are infected inhabitants. Thus, control of transmission should involve measures aimed at (1) reducing the consumption of improperly-cooked fish, and (2) reducing the level of environmental contamination with *O. viverrini* eggs, e.g. contamination of water. Specific measures of control, i.e. chemotherapy and snail control, generally contribute little or no impact on other health conditions, though effective chemotherapy will have a direct disease-preventive role in reducing quickly the prevalence and intensity of infection, morbidity and consequently transmission. Non-specific measures involve improved standards of living and health conditions, which may be more acceptable environmentally than chemical control of snails. Even with effective non-specific measures and snail control, the reduction in prevalence and intensity of infection is very slow, and reduction in morbidity and disease becomes secondary.

In treating *O. viverrini*-infected patients, many drugs have been tried, but they are neither effective nor safe, and these include chloroquine,[75] entobex,[76] dehydroemetine,[77] hetol,[78] amopyroquin,[79] mebendazole,[80] and albendazole.[81]

Recently, praziquantel has been found to be an effective drug on hospitalized patients at 25 milligramme per kilogramme (mg per kg) body weight three times daily for one or two days, with a cure rate of 100%,[82] or at 40 mg per kg body weight single dose, with a cure rate of 90.9%.[83] In heavily infected patients, the drug is effective at 50 mg per kg body weight, with a cure rate of 97.0%.[84] A clinical field trial was conducted in Nong Ranya village, Khon Kaen province, where the rate of prevalence was 94%,[27] using 40 mg per kg body weight single dose, which give a cure rate of 79.5%.[85] The drug is safe, with mild and transient side effects, such as dizziness, abdominal discomfort and diarrhoea (Fig. 12).[82-85]

Control of opisthorchiasis through snail control may not appear practical because of the widespread distribution of the snails and their ability to survive in very unstable habitats. Furthermore, minnows are an important source of animal protein for the villagers, and any use of conventional molluscicides or environmental manipulation that reduces fish populations could have undesirable economic or health effects which must be incorporated into the reckoning of costs and benefits.[36]

A recent field trial, using the integrated methods of control by means of repeated mass chemotherapy, health education and sanitary improvement, was performed for 36 months in three rural villages in northeast Thailand. The parasite was successfully controlled, with reduction in prevalences from 64.4% (village 1) and 56.8% (village 2) to 4.4% and 5.3%, respectively, whereas prevalences in the control village (village 3) varied slightly during the first two years (65.2% and 71.2%) but decreased considerably in the third year (44.2%) of the study period.[86]

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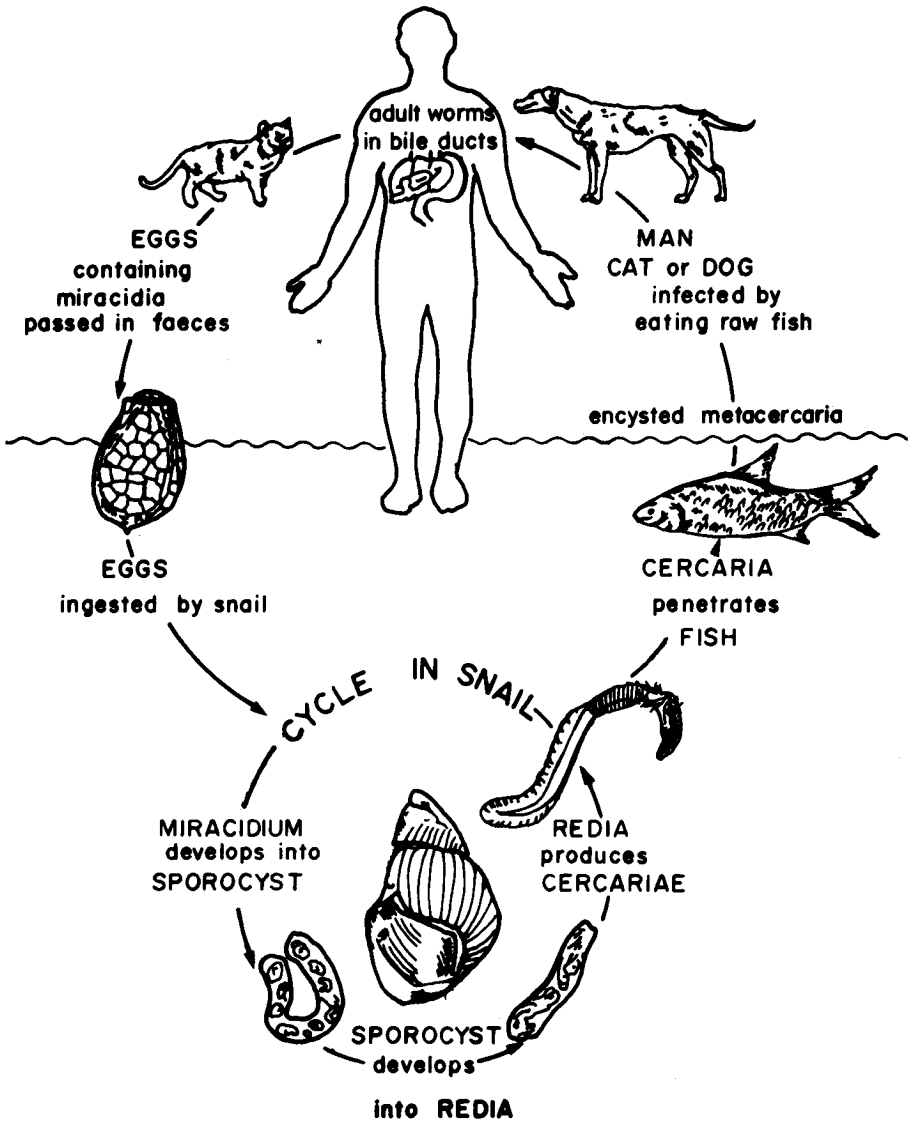

Fig. 1 Showing the life cycle of *Opisthorchis viverrini.*

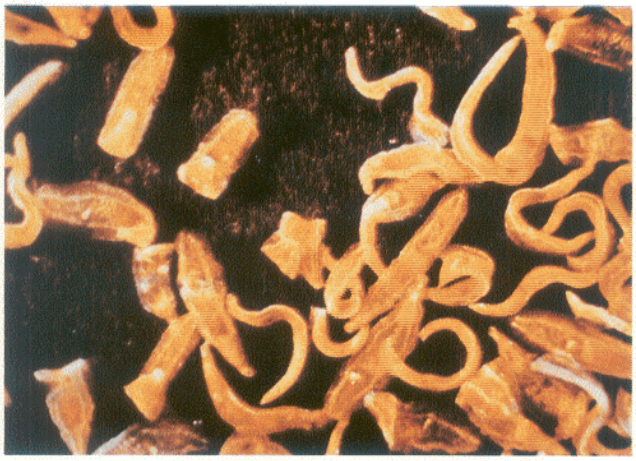

Fig. 2 Showing adult of the human liver fluke.

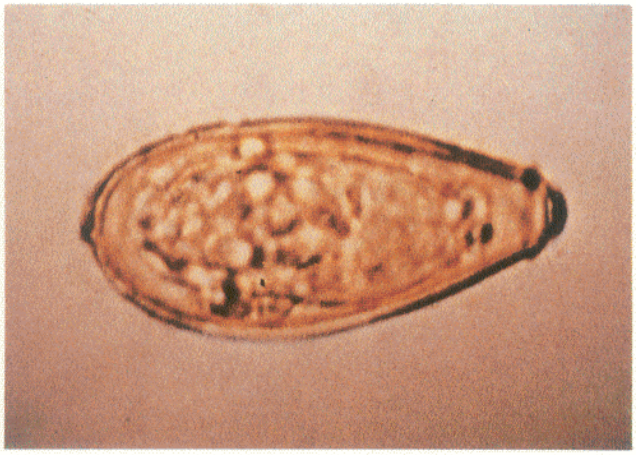

Fig. 3 Showing egg of the human liver fluke.

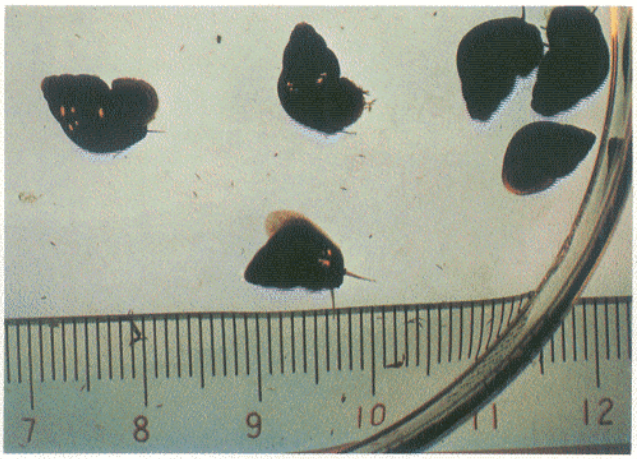

Fig. 4 Showing the snail intermediate host of *Opisthorchis viverrini*.

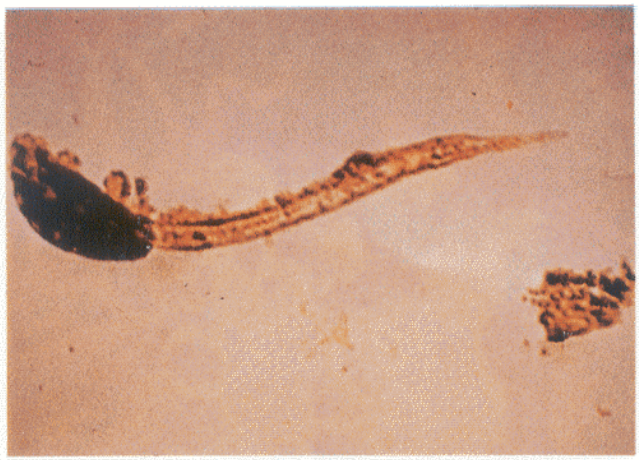

Fig. 5 Showing cercaria of *Opisthorchis viverrini.*

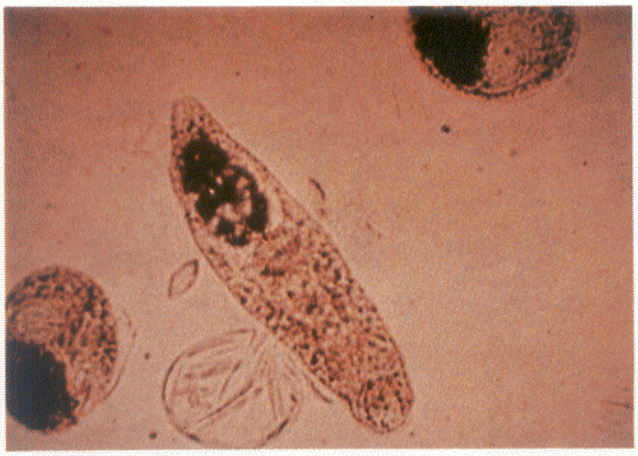

Fig. 6 Showing metacercariae of *Opisthorchis viverrini.*

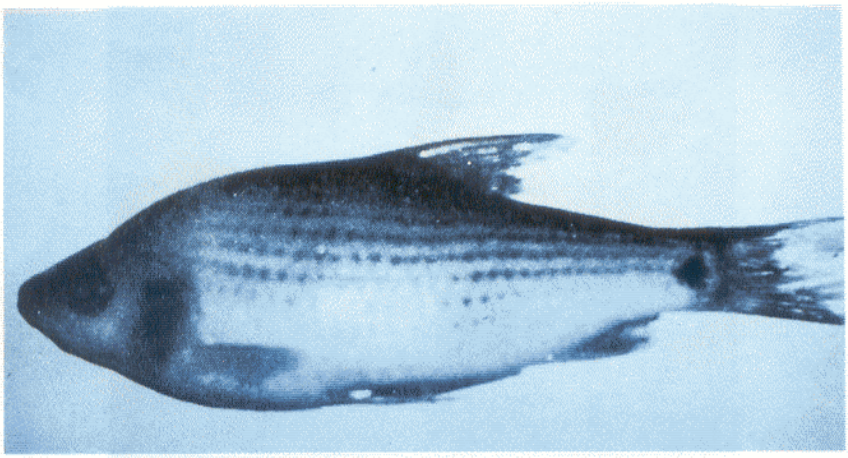

Fig. 7 Showing *Cyclocheilichthys apagon,* a fish intermediate host of *Opisthorchis viverrini.*

Fig. 8 Villagers are eating the raw fish dish, "Koi-pla".

Fig. 9 A shallow water reservoir where snails and fish are found abundantly.

Fig. 10 Showing core sampler for studying population changes of the snail intermediate host.

Fig. 11 A villager catches fish in a water reservoir.

Fig. 12 Treating *Opisthorchis viverrini* - infected people with Praziquantel.

บทคัดย่อ

โรคพยาธิใบไม้ตับที่คนในประเทศไทยเป็นกันมากเกิดจากพยาธิใบไม้ตับชนิด *Opisthorchis viverrini* ซึ่งมีผู้ป่วยด้วยโรคนี้ประมาณเจ็ดล้านคนในภาคตะวันออกเฉียงเหนือและภาคเหนือของประเทศ พยาธิใบไม้ตับนี้มีหอยและปลาน้ำจืดเป็นโฮสท์กึ่งกลางตัวที่หนึ่งและตัวที่สองตามลำดับ คนติดเชื้อพยาธิใบไม้ตับได้โดยรับประทานปลาดิบ เช่น "ก้อยปลา" ผู้เขียนได้รวบรวมผลการทดลองไว้ ณ ที่นี้เพื่อเป็นแนวทางสำหรับผู้ที่สนใจหรือผู้ที่เริ่มจะทำวิจัยในด้านนี้