

CHAPTER 4

DISCUSSION

Metacercaria infection in cyprinoid fish

Among the four species of cyprinoid fish from natural habitats, *Puntius leiacanthus* (ปลาตะเพียนทราย) appeared to be the most common species available in the markets in the five districts of Chiang Mai province during the period of study. However, this kind of fish was rarely cultured in fish farms where *P. gonionotus* (ปลาตะเพียนขาว) was predominant, probably due to the demand of consumers. More species of fish could have been examined if an additional survey of cyprinoid fish in natural water resources was carried out. Infection with *O. viverrini* metacercariae among fish from natural habitats was observed in 19 out of 249 (7.6%) *P. leiacanthus* and 5 of 131 (3.8%) *Osteochilus* sp. (ปลาสร้อย) (overall = 5.0%, Table 3). Much lower infection was observed in *P. gonionotus* from fish farms, i.e. 2 out of 247 or 0.6% (overall = 0.6%, Table 4). Most of fish examined were infected with small intestinal flukes, i.e. *Haplorchis* spp. Some metacercariae of *Haplorchis* sp. I were fed to hamsters and recovered worms were identified as *Haplorchis taichui* (data not shown). For *Haplorchis* sp. II and III, although no confirmation for the adult morphology was made, their appearances were comparable to those of *Haplorchis yokogawai* and *Haplorchis pumilio*, respectively.

In addition to the infection rate, the intensity as well as the density of *O. viverrini* infections were also significantly higher in fish from natural habitats (Tables 6-9). Similarly, such differences in degree of infections with the metacercariae of *Haplorchis* spp. were also observed, confirming that higher degree of transmission occurred in natural water resources rather than fish farms. This may be explained due to the fact that the

bodies of natural water resources are easily contaminated by excreta of humans, as observed by Sadun (1955). Sornmani *et al.* (1973) found that the degree of infection of the intermediate and definitive hosts is low in new man-made water reservoirs, much lower than in irrigation villages surrounding them. The presence of metacercariae in fish in fish farms may be attributed by the observation that in all fish farms studied water supply has been taken from irrigation canals, rivers or other water reservoirs (see Figure 11, Appendix C) which could bring the farms infected snails, freed cercariae or even infected fishes. These results may imply that people who consume raw or improperly cooked fish collected from a fish farm would receive a lower risk of getting infection with opisthorchiasis compared with those consuming fish collected from their natural habitats. However, this may not be true in the case of *Haplorchis* spp., especially *Haplorchis* sp. I, since its infection rates were relatively high in both fish farms and natural habitats (about 70%-98%, Table 3, 4).

The infection rates of fish with *O. viverrini* metacercariae were relatively low when compared with the results of previous studies in Chiang Mai. Sujjanun and Thitasut (1971) reported that the infection rates in *P. leiocanthus*, *P. orphoides* (ปลาแก้มช้ำ) and *Esomus metallicus* (ปลาชิว) collected in various areas of Chiang Mai were 60%, 94.4% and 8.4%, respectively. However, the rate in *P. leiocanthus* as found in the present study is comparable to a recent survey by The Department of Parasitology, Faculty of Medicine, Chiang Mai University (unpublished), that 6 out of 70 (8.6%) were infected. Ratanasritong and Kliks (1972) reported the 3.8% infection rate in *P. gonionotus* collected around Chiang Mai areas. However, Eusaeng (1979) found that none of the 30 *P. gonionotus* examined were positive for *O. viverrini*; 100% infection were found in

Cyclocheilichthys apagon (ปลาตะไกร), while all of both fish were infected with *Haplorchis* spp.

The exact reason for such low prevalence of *O. viverrini* metacercaria infection in fish collected from natural habitats in the present study is unclear. It seems unlikely that this is due to the impact of opisthorchiasis control program because the drug used for killing *O. viverrini* is also effective to small intestinal flukes, and consequently a significantly decreased infection in fish with *Haplorchis* spp. would also be seen. One possible explanation is that most fish from natural habitats examined in the present study were collected from the areas that the *Bithynia* snails were very low in density such as in the large rivers, where infected snails were hardly found (Ditrich *et al.*, 1990; Papasarathorn *et al.*, 1980).

High infection of fish with *Haplorchis* spp. rather than *O. viverrini* metacercariae was also previously observed in Chiang Mai by Manning *et al.*, (1971), Eusaeng (1979) and in Laos by Giboda *et al.* (1991). This may be explained due to the differences in the infection rate and the distribution of the snail first intermediate hosts. The snail *Thiara* (= *Melanooides*) *tuberculata*, not *Bithynia* spp., is the intermediate host of *Haplorchis* spp. in northern Thailand with the cercarial infection rate being 11.5% (Kliks and Tantachamrun, 1974) whereas the rate observed in *Bithynia* snails throughout the country was normally lower than 1% (e.g. Thirachantra and Khamboonruang, 1971; Impand *et al.*, 1994). In addition, *T. tuberculata* inhabits a wide variety of water bodies, but *Bithynia* snails are rarely found in strong running water (Kliks and Tantachamrun, 1974).

In northeastern region, the high infection rates ranging from 51 to 100% were found in *C. apagon*, *C. siaja* (ปลาแม่สะแต้ง), *P. orphoides*, *P. leiacanthus*, *P. partipentazona* (ปลาเสือสุมาตรา) and *Hampala dispa* (ปลากระต๊อบ) (Wykoff *et al.*,

1965; Vichasri *et al.*, 1982) (see also Table 1). The infection rates in *P. gonionotus* and *Osteochilus* sp. were as low as in the north, i.e. only 3% and 4% respectively (Wykoff *et al.*, 1965). Perhaps, these fish might be less attractive to the cercariae of *O. viverrini*, or their habitats may be less associated with the *Bithynia* snails than the other species of fish. Definite conclusion needs further investigations.

From the epidemiological point of view, the finding in the present study that most cyprinoid fish were infected with *Haplorchis* metacercariae suggests the possibility that a significant proportion of the human cases in northern region, as diagnosed by stool examination being opisthorchiasis, may be attributed to one of several small intestinal flukes. This is because their eggs are very similar to those of *O. viverrini* when examined under light microscope. Radomyos *et al.* (1984) reported that cases in northeastern region infected with only small intestinal flukes without *O. viverrini* were rarely found. Therefore, it is considered that the finding of this type of eggs in the stool would indicate the presence of *O. viverrini*. However, this may not be always true in the northern region or in Laos where cases with only small intestinal flukes were frequently reported (Kliks and Tantachamrun, 1974; Ditrich *et al.*, 1990).

Metacercariae in raw-fish products

The present study did not provide any evidence that "*pla-jom*" and "*pla-som*" were important in transmission of *O. viverrini*. A number of metacercariae were found in "*pla-jom*", but most of them were completely degenerated although they were incubated for only 3-7 days (Table 10). There appear to be 3 main factors that can cause the degeneration of metacercariae in raw fish products, namely salinity, temperature and time. Kruatachue *et al.* (1982) demonstrated that when the metacercariae of *O. viverrini* had

been incubated for 24 hours in various concentrations of sodium chloride, the infectivity in hamsters decreased from 100% at 0.85% to 2.4% at 6.8%; at 13.6% concentration, no infection occurred. It is possible that salinity has a great effect on the osmotic pressure inside the metacercariae. The hypotonic solution of sodium chloride may cause swelling and rupturing of the metacercariae cysts, whereas the hypertonic solution may cause the shrinkage of the cysts. In addition, they also observed that those incubated at 10°C to 20°C gave the infection rates of 80%, but at 25°C to 40°C the infection rates and the numbers of worm recovered were gradually decreased; however, they found that the infectivity was not affected by pH values (4.4 to 9.4).

The sodium chloride concentration in "*pla-jom*" may be as high as 10%, according to the 1:10 ratio of salt and fish. Normally, "*pla-jom*" must be kept for a few months before being eaten. Therefore, it could be expected that at the end of fermentation time all metacercariae are not viable.

The present study failed to detect any metacercaria of *O. viverrini* or others in "*pla-som*" samples, neither did other previous studies (e.g. Sadun, 1955; Harinasuta *et al.*, 1961). This may not be due to the effect of salinity as a small amount of salt is normally added in this food. It might be that cyprinoid fish were seldom used in preparing such food, due to their small amount of meat. However, the infectivity of metacercariae in "*pla-som*" may be observed experimentally.

Another kind of food which has been considered to be important in transmission of *O. viverrini* is "*pla-ra*", one the most common food throughout the country; so far, however, several attempts have failed to show the evidence (e.g. Sadun, 1955; Harinasuta *et al.*, 1961). Tesana *et al.* (1986) observed the infectivity of their laboratory-made "*pla-ra*" containing *O. viverrini* metacercariae. They found that at 10% sodium chloride

and for a few hours of exposure, the infectivity was decreased by 50%. Unfortunately, no observation was made for a longer fermentation time. The sodium chloride concentration in "*pla-ra*" sold in the markets which ranged from 7.7-20.7% (Hongthongdaeng, 1979) and its fermentation time, which is normally a few months at room temperature, are very likely to be lethal to the metacercariae.

Therefore, it is very unlikely that people will acquire opisthorchiasis by eating food produced from fermented fish, with a proper salt concentration and a normal incubation time. It is evident, however, that some of "*pla-ra*" sold in the markets may be freshly made or mixed with fresh cyprinoid fish. Some people believe that the metacercariae, if any, might be viable but this still needs evidence from further investigations.

At present, a large range of technologies are currently applied in food processing as summarized in Table 11. However, treated fish may not be convenient or preferable for people to consume. Further attempts should be made to change the eating habits of people, especially young generation.

Table 11. Processing conditions under which inhibition of infectivity of food borne *O. viverrini* metacercariae has been observed.

Process	Parasite	Processing parameters		References*
		temperature	time	
heating	free metacercariae	50°C	5 hrs	(1)
		70°C	30 min	(1)
		80°C	5 min	(1)
acidity	free metacercariae	concentration	time	(1)
		commercial vinegar	4%	1 hr
acetic acid		4%	1.5 hrs	(1)
lactic acid		4%	1.5 hrs	(1)
citric acid		4%	1 hr	(1)
salting (NaCl)	free metacercariae	concentration	time	
		0.9%	10 days	(1)
		10%	3.6 hrs	(1)
		20%	1.2 hrs	(1)
		30%	1 hr	(1)
	metacercariae in fish	13.6%	24 hrs	(2)
irradiation	metacercariae in fish	absorbed dose		
		0.1 KGy	-	(3)
freezing	metacercariae in fish	temperature	time	
		-10°C	5 days	(4)

* (1) Waikagul (1974); (2) Kruatachue *et al.* (1982); (3) Sornmani *et al.* (1993); (4) WHO (1995)