CHAPTER V
DISCUSSION AND CONCLUSION

5.1 Discussion

High prevalence of fish-borne zoonotic trematodes (FZT) including *Opisthorchis viverrini* and several species of minute intestinal fluke (MIF) in human reported in Lao PDR reflect high transmission of these trematodes in this country (Chai et al., 2005b; Sithithaworn et al., 2006; Stensvold et al., 2006). The highest prevalence rates in human were found in central and southern parts, reaching levels of up to 60% (Sayasone et al., 2007; Sayasone et al., 2009). In addition wild 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; Scholz et al., 1990). Recently, metacercariae of *O. viverrini*, *Haplorchis taichui*, *Haplorchis yokogawai* and *Centrocestus formosanus* were detected in the fish from Savannakhet and Vientiane, Lao PDR (Rim et al., 2008).

The data we presented here were form the first control trial to reduce contamination of FZT in aquaculture farms in Lao PDR based on a longitudinal study design. In this study, *H. taichui* metacercariae were highly common in most species of culture fish in the hatcheries farms studied.

In Lao PDR, *O. viverrini* a member of FZT was reported in wild fish in Savannakhet and Vientiane particularly Nam Ngum wetlands (Saijuntha et al., 2007). In the previous study, a survey of 13 fish farms in Vientiane province revealed that fingerlings from 2 farms were positive for *O. vivverini* and other FZT metacercariae (unpublished). In this study, 8 out of 9 species of fish harbored FZT namely *H. taichui*, *Centrocestus* sp., *O. viverrini* and *H. pumilio*. Generally, the situation of FZT in fish farms in Lao PDR is similar to those in Vietnam and possibly Thailand since similar fish species are used for stocking in aquaculture particularly carps.

Implementation of preventive measures against FZT in the fish pond in this study gave positive effect in some species of fish i.e. silver carp, mrigal, rohu as well as silver barb. But little or no effects of these measures in the other 3 species of fish i.e. common carp, grass carp and bighead carp. There are several possible explanations for this finding – Firstly, since the fish pond is polyculture system in which 9 species are kept in the same pond and thus interaction/competition among fish and or selection of cercariae by fish species may influence the infection outcome. Secondly, most of the fry used here were originated within the farm and already infected with high percentage in some species (40-60%) thus to reduce the prevalence and intensity of FZT is therefore unlikely because the metacercairae are viable for almost one year. The third explanation that in Lao-Viet farm where the majority of data were generated is that at month 3 of the experiment, there was heavy rain and the barrier of the treatment pond was damaged which may allow water and snail to come into the treatment pond. However, overall, prevalence of infection in 3 species of fish was significantly reduced compared with the untreated control. A previous control trial of silver barb in Chiangmai province also yielded unsatisfactory results (Khamboonruang et al., 1997). It appears that the pond preparation and sources of FZT needed better and more stringent control and this requires analysis of cost-effectiveness at the practical level.

As the fish are kept longer, the trends of increasing infection in both prevalence and intensity of infection for example in case of *H. taichui* with age or size of fish are observed. To avoid this problem, a shorter duration for stocking fish by using fast growing species may help to control and reduce FZT. In particular, resistant species such as red finned pecu and nile tilapia are less susceptible to FZT should be encouraged for stocking. In case of susceptible species such as silver barb which is native and fast growing and well accepted species, requires a reconsideration of fishery policy or promotion. A selection of fish species with resistant breed or genotype to FZT should be searched for and encourage stocking of FZT-free fish to farmer. (Phan et al., 2010a)

The evidence of snail infection with parapleurolophocercous cercariae which may lead to both *H. taichui* and *H. pumilio* metacercariae in fish indicated direct roles of these

snails (*Melanoides tuberculata* and *Tarebia granifera*) as the sources of pond contamination. In addition, the cercariae may also come with replenished water into the fish pond. Although the snails were abundant in feeding canal outside the fish ponds, it was not observed in the treatment pond in LV farm in this study. During flooding, however, both snail and cercariae are likely to passively enter the fish pond. Observed data indicated that the infection rates in snails were relatively high (7-10%) thus there is high possibility of having infected snails in the fish pond. Moreover, FZT infections in snails were consistently found at all sampling points from month 0 to 5 i.e. in LV farm. Direct contamination of free swimming cercariae as opposed to invading snail was reported in Vietnam and the authors suggested that cercaraie contamination of the fish pond originated from replenished river water (Skov et al., 2009). Thus cercariae shed from the snail within the fish pond may not always be the source of FZT metacercaria appeared in fish. This not the case in this study for *H. taichui* and *H. pumilio* but for others such as *Centrcestus* sp. it is not clear.

Animals (mainly cats) and humans in the farm, particularly LV farm, were infected with FZT namely *O. viverrini* and MIF. Couple with the presence of snail intermediate hosts (*M. tuberculata* and *T. granifera*) in the ponds and natural canal, FZT can contaminated the ponds and spread to fish. Similar data of infected animal hosts with FZT were recorded in Vietnam (Anh et al., 2009a, 2009b). The roles of these animal hosts raise concern for FZT control in human (Phan et al., 2010b). Whether human and animals share the same genotypes of FZT or not as those in *schistosoma japonicum* in the Philippines (Rudge et al., 2008) remain to be investigated.

The presence of *O. viverrini* in silver barb in SSPP farm came as no surprise since a previous survey by Sithithaworn et al (unpublished) has demonstrated that *O. viverrini* were detected (by PCR) in 4 out of 14 farms in Lao PDR. Occurrence of *O. viverrini* was found at month 1 and 4 in fingerling silver barb but not at subsequent samplings. Silver barbs have been reported to have natural infection with *O. viverrini* as well as other FZT (Rim et al, 2008) and in Thailand (unpublished, Pitaksakulrat). This is the second study showing that *O. viverrini* was found in culture fish in Lao PDR and hence posed

considerable public health concern. A similar study in Vietnam also indicated that *C. sinensis* in addition to other FZT were presented in culture fish in northern part of the country (Phan et al., 2010a).

In this study, metacercariae were identified by morphological characteristics and 3 known species were encountered namely *O. viverrini*, *H. taichui* and *Centrocestus* sp. In case of *H. pumilio*, the key characteristics (pattern of spines at vantrogenital complex) were not easily observed and hence they are called as unidentified or unknown metacercariae. These unknown metacercariae from each species of fish were then pooled and kept for PCR identification using specific DNA primers (Wongratanacheewin et al., 2001; Parvathi et al., 2007; Sato et al., 2009). As shown in Table 4.1 and 4.2, within the unknown metacercariae, *O. viverrini*, *H. taichui*, *H. pumilio* and *Centrocestus* sp. were identified. No *C. sinensis* was found. In LV farm, *H. pumilio* was found in red finned pecu which was not observed by the conventional method. Most importantly, metacercariae of *H. pumilio* were discovered in 4 species of fish (siver barb, mrigal, grass carp and red finned pecu). In SSPP farm, *Centrocestus* sp. metacercariae were found in silver barb by PCR in addition to *O. viverrini* and *H. taichui* by the conventional method.

In case of cercariae, parapleurolophocercous cercariae were found in *M. tuberculata* and *T. granifera* by morphological identification. With the aid of PCR, cercariae typel (*H. taichui*) and type 2 (*H. pumilio*) were confirmed. Our result (table 4.3) showed that *H. pumilio* was found only in *M. tuberculata* while *H. taichui* was found only in *T. granifera*. Whether these snails are susceptible to both *H. taichui* and *H. pumilio* as observed in Vietnam (Dung et al., 2010) is not known and deserved further investigation. The utility of PCR for identification and confirmation of FZT species at metacercarial and cercarial life stages is clearly of particular benefit in case of a few worms are available and have no access for laboratory animal experiment. The PCR–based diagnosis have also been applied in human for coprodetection of *O. viverini* DNA (Duenngai et al., 2008).

5.2 Conclusion

1. This research is the first control trial of FZT at fish farm level in Lao PDR.

2. The FZT detected in fish farms in Lao PDR were *H. taichui*, *H. pumilio*, *Centrocestus* sp. and *O. viverrini*.

3. The FZT control trial at farm level has demonstrated reductions in prevalence of *H. taichui* infection in 3 out of 7 species of fish.

4. Trends of reduction in intensities of *H. taichui* infection were observed in 4 species of fish but not reached statistical significance.

5. *H. taichui* infection (both prevalence and intensity) in fish were related to fish body size (weight, length and width). This pattern of infection indicated that transmission of FZT occur in the fish pond as well as those original infection in fish fry.

6. Silver barb (*B. gonionotus*) was the most susceptible to FZT (mainly *H. taichui*, *H. pumilio*, *Centrocestus* sp. as well as *O. viverrini*) and did not significantly affect by the control measures. But significant reductions in intensity of *H. taichui* were observed at month 5 and 6 between treated and untreated ponds.

7. Nile tilapia and red finned pecu are more resistant to FZT infection and from the FZT perspective they may be suitable for aquaculture in Lao PDR.

8. Among unknown or unidentified metacercariae, when analyzed by specific PCR, *H. pumilio* was discovered in several species of fish.

9. The finding of *O. viverrini* in silver barb in one of the studied farm indicated the roles of aquaculture as source of FZT in human and hence this has public health implication.

10. Further improvement of the control trial should aim at using FZT-free fish fry so that the impact of the control measure can be effectively evaluated.