CHAPTER 5

DISCUSSIONs

Epidemiology of *Haplorchis taichui* in Chiang Mai Province

Haplorchis taichui infections are commonly found in the northern and northeastern regions of Thailand. Several species of cyprinoid fish have been reported as the second intermediate hosts (Kliks and Tantachamrun, 1974; Srisawangwong *et al*., 1997b; Namue *et al*., 1998; Waikagul, 1998; Sukontason *et al*., 1999; Wongsawad *et al*., 2000). In the present study, 15 species of cyprinoid fish were found infected with *H. taichui* and other heterophyid metacercariae, namely *Centrocestus caninus*, *Haplorchis pumilio* and *Haplorchoides* sp. Among the metacercariae, *H. taichui* had the most and were found throughout a year-round survey. Obviously, *Haplorchoides* sp. was mixed-infected and was predominantly found in several species of fish. However, this species is mostly found in fish scales and adults parasitize the intestines of catfish (Waikagul, 1998). From this study, *H. taichui* metacercariae were mainly recovered from the muscles of fish. Previously, they were mainly found in the caudal fin (Sukontason *et al*., 2001a). Probably be metacaercariae are mostly found in the muscles of the base of the caudal fin. The metacercariae are predominantly localized in muscles from both study sites, while the fewest were found in the gills. It should be recorded that *H. taichui* metacercariae were also commonly found in fish heads just as in the muscles.

The minute intestinal fluke's egg is similar in shape and size to that of the liver fluke, *Opisthorchis viverrini* (Manning *et al*., 1971; Tesana *et al*., 1991; Radomyos *et al*., 1998). In addition, the general life cycle of *H. taichui* is parallel to

Opisthorchis spp. (Cheng, 1964). Mixed infections with *O. viverrini* and heterophyid flukes have been reported from many areas (Radomyos *et al*., 1998; Sukontason *et al*., 1999; Chai *et al*., 2005). Therefore, it is difficult to identify parasite species from fecal eggs. This finding differs from previous study which revealed that most of eggs found in man were *O. viverrini*, while no *H. taichui* were found (Chiang Mai Provincial Public Health Office, 2001). However, investigations of *O. viverrini* metacercariae in the second intermediate host revealed a low infection rates (Ratanasritong and Kliks, 1974; Eusaeng, 1979; Poolphol, 1995; Sukontason *et al*., 1999). The present results are similar to previous reports that *H. taichui* has the highest incidence in northerners (Pungpak *et al*., 1998; Radomyos *et al*., 1998; Chuboon and Wongsawad, 2003). The distribution of food-borne trematode infections depends on many factors such as the relationship between the host, parasite, environment and traditional food habits of people (WHO, 1995). Humans are not only the source of this heterophyid fluke's egg, since fish-eating animals can also be involved in their life cycles (Faust and Nishigori, 1926; Pearson 1964; Ditrich *et al*., 1990). In this study, *H. taichui* showed broad ranges of host specificity because it can infect various species of cyprinoid fish. Food-borne trematode infection can be assessed by the prevalence of infection. Chom Thong District had higher prevalence and intensity of metacercarial infection in fish. It is possible that this area is one of the high-risk areas in Chiang Mai Province. In addition, Thai traditional dishes such as *plasom* and *labpla* are believed to be the source of infection to people. These raw-fish dishes cannot induce degeneration of the contaminated metacercariae in a short period (Sukontason *et al*., 1998; Wiwanitkit *et al*., 2002). In poorly cooked fish, metacercarial cysts can survive for up to 7 days in salted fish (Pica *et al*., 2003).

There is a tendency that the number of worms infecting man will increase because of acquiring the infections from eating raw-fish dishes. Therefore, eating habits of the local people should be concerned for the better education about consuming raw fish.

Life history of *Haplorchis taichui* in various hosts

The recovery rate of worms infecting chicks and mice showed the susceptibility of the hosts. Chicks can harbour this worm for long time and with a higher incidence of infections than mice. Probably the definitive host of this fluke is birds more than mammals. Recovery of *H. taichui* were found in experimental chicks from day 1 to day 48 PI and mice from 1-12 day PI. On the third day, eggs were found in the uterus of worms in chicks, as the same as in the experimental mice. According to a previous study *H. taichui* adults rapidly develop in mice for 3 days PI (Sukontason *et al*., 2001b). In this study, parasites invaded in the jejunum during days 1-2 PI, but on day 3 PI and later they were mostly found in the ileum and rapidly decreased after day 16 PI. *H. taichui* and *H. pumilio* invade the upper part of the small intestine during the first period of infection, but when they mature they are more common in the middle and lower parts (Faust and Nishigori, 1926). This is because the flukes become gradually excreted into the lumen of the intestinal canal along with mucus and other exudates, then reattach further down. In addition, one adult was found in the duodenum and one metacercaria in the ileum. It is suggested that it was excysted in the duodenum, but the micro-environment of this area is not appropriate for the growth of this worm. Metacercaria in the ileum will be excysted or excreted with the feces of the host. Excystation depends on some combination of external factors from the host and internal factors from the parasite along with the excystation site and complexity of the cyst wall (Asanji and Williams, 1974; Fried, 1994). Moreover, *H. taichui* survived in chicks until day 48 PI and a good yield of adults can be obtained until day 16 PI. In mice the best yield was obtained only during the first 5 days PI (Sukontason *et al*., 2001b). Probably the micro-environment in chicks was better than that in mice for *H. taichui*, as is similar to another heterophyid fluke, *Stellantchasmus falcatus* (Wongsawad *et al*., 1998). On the other hand, Chai (1979) suggested that the low worm recovery rate of *Metagonimus yokogawai* was possibly due to their short life span and smaller size of mature worms. However, this study presented a better yield and a higher recovery rate in chicks than mice, since the life span of *H. taichui* in chicks is longer than that in mice. Therefore, chicks can be used as a suitable definitive host for *H. taichui* to collect adult worms.

Intermediate host infection of *H. taichui* was performed in laboratory conditions. The snail, *Tarebia granifera*, served as the first intermediate host. The larval stages of worms develop rapidly and mature parapleurolophocercous cercariae can found in seven weeks. Previously, cercacariae of *H. taichui* developed in *T. granifera* in six weeks from Formosa (Faust and Nishigori, 1926). The results showed that *T. granifera* is one of the first intermediate hosts of *H. taichui*. According to studies in Hawaii and Laos, *H. taichui* can infect *T. granifera* (Faust and Nishigori, 1926; Martin, 1958; Giboda *et al*., 1991). Obviously, parapleurolophocercous cercariae was found belonging to the family Heterophyidae, which also found in the other species such as *Haplorchis pumilio* (Sadd and Abed, 1995), *Stellantchasmus falcatus* (Martin, 1958; Kliks and Tantachamrun, 1974), *Centrocestus unequiorchalis* (Saad, 1994). In the metacercarial stage, mature cysts are found at 6 days post-infection in the cyprinoid fish (*Barbodes gonionotus*). Kliks and Tantachamrun (1974) reported that parapleurolophocercous cercariae shedding from naturally infected *Melanoides tuberculata*, it can develop in *Cyprinus carpio* at 5 days PI. However, the second intermediate host has many species of the families Cyprinidae, some species of the family Siluridae and Colitidae (Witenberg, 1929; Yamaguti, 1958; WHO, 1995).

Tegumental surface of metacercariae and adults of *Haplorchis taichui*

The surface structure of this fluke has been studied to understand their morphological and functional characteristics. In metacercariae of *H. taichui*, the number of multi-pointed spines were digitated with 3-11 points according to previous reports and was covered with 3-11 points (Sukontason *et al.*, 2000a). This study used a scanning electron microscope to study the surface ultrastructure of the adult *H. taichui*, which is covered with scale-like, multi-pointed 3-12 points. In a previous report the number of points was 5-12 (Scholz *et al.*, 1991). Developmental changes in the tegumental spines have been well known in many species of trematodes. The differentiation of simple, multi-pointed spines during the parasite's development has also been reported in some heterophyid flukes such as *Metagonimus takahashii* (Chai *et al.*, 2000) and *Heterophyopsis continua* (Hong *et al.*, 1991). Small numbers of fewer pointed tips in juvenile may supportive for locomotion of worms and migration during development to adults. The more pointed and enlarged spines of adults help them to maintain good contact with the intestinal wall and prevent them from being passed in feces, and also help flukes to adjust to the microenvironment in the host (Bennett and Threadgold, 1975; Srisawangwong *et al.*, 1989; Chai *et al.*, 2000; Sukontason *et al.*, 2000a). The present study found four types of sensory papillae: type I, round swellings of the tegument; type II, ciliated dome-shaped; type III, non-ciliated dome-shaped and type IV, button-shaped papillae. Ciliated and button-shaped papillae which are distributed on the tegumental surface act as tactile receptors while the swellings of tegument and non-ciliated dome-shaped papillae can act as pressure receptors (Dangprasert *et al.*, 2001). Moreover, the swellings of tegument papillae situated on the lip of the oral sucker may be responsive to pressure and sense contact during suction to the host's tissue (Apinhasmit *et al.*, 1993). There were numerous ciliated papillae around the oral sucker which suggests that the oral sucker exerts a force to attach to the intestinal mucosa and actively probe the host's environment for feeding (Hong *et al.*, 1991). Anyhow, ciliated sensory papillae have been found in many species of trematodes (Bennett, 1975; Srisawangwong *et al.*, 1989; Chai *et al.*, 2000; Sukontason *et al.*, 2000a). *H. taichui* has numerous ciliated papillae in a single or group bilaterally symmetrical arrangement, especially around the oral sucker and anterior region. The cilium is extended through the tegument and connected to nerve fibers. Thus suggests that it may function as tango-receptor, rheo-receptor, mechano-receptor, or chemo-receptor (Chai *et al.*, 1992; Chai *et al.*, 2000; Hong *et al.*, 2004).

Tegumental changes on the adult of *Haplorchis taichui* after treatement with niclosamide

This study revealed that niclosamide is highly effective in killing adult of *H. taichui in vitro* at a low concentrations. All of worms lost their mobility after 1 hour in 0.01μg/ml and after 30 minutes in 0.1μg/ml of niclosamide solution. This finding is similar to the effects of praziquantel on some trematodes such as *Fibricola seoulensis* (Lee, 1985) and *Paragonimus westermani* (Lee *et al.*, 1987). It is suggested that niclosamide can kill intestinal flukes in high efficiency and can cure

Heterophyidiasis. Anyhow, niclosamide has been reported to cure some fluke infections such as *Metagonimus gokogawai* (Rim, 1975) *Fasciolopsis buski* (Suntharasamai *et al.*, 1974) and *H. taichui* (Sukontason *et al.*, 2000b).

This study has shown that niclosamide causes progressively severe disruption to the tegumental surface of *H. taichui* with longer exposure times. After 30 minutes to 6 hours of exposure in 0.01 μg/ml and 30 minutes to 1 hour in 0.1, 1, and 10 μg/ml, morphological changes indicated in the anterior region, especially around the oral sucker and ventrogenital sac. Variously sized blebs developed in the area between the oral sucker and ventrogenital sac. After 6 hours of 0.1, 1.0 and 10 μg/ml concentrations, more severe disruption was evident in the posterior region. The movement and feeding are disrupted since the drug penetrates the pharynx and the early gut, which causes anterior changes in the early and the posterior region more severely with longer exposure. The more rapid anterior blebbing is probably due to rapid absorption of niclosamide. It probably be expected that when the fluke has normal movement the drug can diffuse to the posterior end of the gut by oral uptake. With the longer treatment periods, flukes become less active and finally become immobile. By comparison, the more extensive posterior damage by this drug is due to its accumulation in the posterior region of the gut. The results support the fact that oral ingestion of niclosamide is one route of entry into the fluke. This agrees with reports on some fasciolicides (Meaney *et al.*, 2004; 2005). In addition, the rapid tegument disruption observed *in vitro* is due to more direct contact with the drug. There is a more rapid effective of this drug to the parasite via oral uptake where flukes are quickly paralysed and rapidly killed (Fairweather *et al.*, 1984; McKinstry *et al.*, 2003).

Regional differences in tegumental disruption were observed on treated flukes. The dorsal surface has similar damage as the ventral surface and the posterior region has more severe damage than the anterior part. This study is similar to the effects with praziquantel (Apinhasmit and Sobhon, 1996), albendazole sulphoxide (Boochanan *et al.*, 2003), 5-chloro-2-methylthio-6-(1-naphtyloxy)-1-H-benzimidazole (Rivera *et al.*, 2004; 2005). The reasons of regional differences in damage may be due to tegumental structure, route of drug uptake, and drug metabolism (McKinstry *et al.*, 2003). The drug is more rapidly absorbed when the fluke is contact at earlier exposure times, causing damage with variously sized blebs between the oral sucker and ventrogenital sac. The anterior region is less damaged than posteriorly because this area is densely covered with larger spines while the posterior region is covered with smaller spines.

Surface blebbing and swelling of the tegument were observed after short exposure times. McKinstry *et al.* (2003) suggested that it is an initial stress reaction by the fluke to replace the surface membrane. Previous reports have been described the effects of other anthelmintic drugs (Lee *et al.*, 1987; Stitt and Fairweather, 1993; Apinhasmit and Sobhon, 1996; Meaney *et al.*, 2002; 2003; Buchanan *et al.*, 2003). Blebbing and swelling are early changes leading to loss of the tegument. Anthelmintic drugs can penetrate deeper-lying tissue, causing more severe damage to the flukes. The swelling of the tegument and blebbing, then rupturing and lesioning are similar to reports using praziquantel (Lee, 1985; Lee *et al.*, 1987; Apinhasmit and Sobhon, 1996). The swelling of the tegument is probably due to the disruption of the ion pumps on the apical plasma membrane. This action decreases production of ATP in the mitochondria and adversely affects the ion pump, leading to the influx of water and swelling of the tegument (McKinstry *et al.*, 2003). When tegumental surface disruption becomes more severe, transport is disrupted throughout the syncytium so the plasma membrane cannot be replaced quickly enough to prevent separation of the tegument, leading to complete loss of the syncytium. Drug exposure to the basal lamina and creates small holes, then penetrates the internal tissues, causing greater lesion damage to the fluke (Meaney *et al.*, 2001). In this study, the blebs were formed on the tegumental surface of treated flukes. The loss of the apical plasma membrane and spine sockets occurred on both surfaces. Surface destruction was more pronounced posteriorly than anteriorly on both surfaces. Numerous small holes developed posteriorly while there were fewer anteriorly.

TEM observations confirmed the internal structure damage to this worm after exposure to niclosamide solution in various concentrations. Surface blebbing, vacuolization, and accumulation of secretory bodies beneath the apical plasma membrane and with their accelerated release occurred. Later, open bodies appeared (Rogan and Threadgold, 1984). A stress reaction is typical following immune attack and drug action (Fairweather *et al.*, 1986; Skuce and Fairweather 1990; Stitt and Fairweather 1993). The mitochondria become swollen and vacuoles appear in the syncytium, indicating severe metabolic disruption of the parasite's tissue (Meany *et al.*, 2004). These changes become progressively more severe with longer exposure time, where cells show disorganization and necrosis. Similar changes have been observed after treatment with closantel and DAMD (Skuce and Fairweather, 1990; Meany *et al.*, 2004). The swelling of the basal infolds could have an osmotic basis due to impairment of energy-dependent ion pumps, as could the changes to the mitochondria. These changes induce the paralysis of the fluke (Fairweather *et al.*, 1984; Skuce and Fairweather, 1990). The severe swelling of the basal infolds is evident after 24 hours in 0.01 μg/ml, after 6 hours in 1.0 μg/ml and 1 hour in 10.0 μg/ml. This change maybe a precursor to separation of the syncytium from the basal lamina. In addition, this drug can disrupt and detach the parasite's tegument exposing the basal lamina and cell organelles. Niclosamide has a disruptive effect on the tegument by direct contact or due to oral uptake. These findings suggest that the flukes exposed to niclosamide have all tissue damaged. This study indicates that niclosamide can cure *H. taichui* infection in high efficacy. This drug can penetrate all fluke's tissue causing both external and internal damage.