

## CHAPTER 6

### CONCLUSION

*Haplorchis taichui* metacercarial infections in fish were investigated in Chom Thong and Mae Tang Districts, Chiang Mai Province during November 2001 to October 2002. Six hundred and seventeen cyprinoid fish of 15 species were found infected with *H. taichui* metacercariae. The prevalence of infections were 91.70% (266/290) and 83.80% (274/327), with the mean intensities of 242.93 and 107.44 metacercariae/fish in Chom Thong and Mae Tang Districts, respectively. Both localities showed the high prevalence and intensity of metacercarial infection in fish. These areas are high-risk areas of *H. taichui* infection in Chiang Mai Province. The eating and cooking habits of the local people should be changed with better education about consuming raw fish.

The life history of *H. taichui* was examined in the various hosts under laboratory conditions. The adult worms can develop in the definitive host for 3 days PI in mice and chicks after being infected with metacercariae. Eggs were first observed 7 days PI in mice and 9 days PI in chicks from fecal samples. Embryonated eggs were ingested by the first intermediate host, a freshwater snail, *Tarebia granifera*. After 10 days PI sporocyst and young rediae developed in the digestive tract of the snail. This snail was infected with the larval stage, two generations of rediae, and cercariae develop after 49 days PI. The second intermediate host is a freshwater fish, *Barbodes gonionotus* which infected with cercariae. Encysted metacercariae developed on day 1 and rudimentary genital organs of metacercariae can be observed on day 3 PI. Metacercariae become fully developed after 6 days PI and rudimentary genital organs were observed.

*H. taichui* rapidly develops in various hosts in laboratory conditions. Moreover, bird and mammals can serve as definitive host, causing wide distribution and increased infection rates in the endemic areas. This study found that the complete life cycle of *H. taichui* in the laboratory about 58 days. Adult worms were found on day 3 PI in mice and the egg could be observed in feces on day 7 PI after being infected with metacercariae, while adults were found on day 3 PI in chicks and the egg in feces could be observed on day 9 PI after being infected with metacercariae.

In excysted metacercariae, the body surface is covered with scale-like, multi-pointed tegumental spines. Tegumental spines were digitated into 3-11 points. Adult worms 7 days PI were similar to metacercariae. The tegumental spines were more complex than metacercariae. The digitated spines had 3-12 points. The dorsal surface showed a prominent Laurer's canal which is located in the anterior 4/5 on the left side of the body. Four types of sensory papillae are present throughout the body: type I, round swellings of tegument; type II, ciliated dome-shaped; type III, non-ciliated dome-shaped; and type IV, button-shaped papillae. The more pointed and enlarged spines help adults to maintain a good contact with the intestinal wall and prevent them from being passed in feces. Numerous ciliated papillae around the oral sucker can help the worm attach to the intestinal mucosa and actively probe the host for feeding.

The effect of niclosamide on the tegument of adult *H. taichui* exposed *in vitro* was observed by scanning electron microscope. *H. taichui* adults were incubated in Tyrode's solution containing 0.01, 0.1, 1.0 and 10 µg/ml of niclosamide for 30 minutes, 1, 6, 12 and 24 hours. In 0.1 µg/ml of niclosamide, all worms died after 1 hour of exposure. The tegumental changes to the flukes were determined by SEM.



Swelling and blebbing of the tegument was observed on both surfaces, ventral and dorsal side. After long periods, extensive swelling and blebbing of the tegument became more severe and some regions lost the apical plasma membrane. These changes consisted of bleb formation followed by rupture and loss of the apical plasma membrane, while empty spine sockets developed and small holes penetrated the basal lamina. The surface destruction was more pronounced posteriorly than anteriorly on both surfaces. The internal structure damage of treated flukes was determined by TEM. Surface blebbing, vacuolization beneath the apical plasma membrane, swollen mitochondria, and vacuoles appear in the syncytium, indicating severe metabolic disruption of the tissue. The severe swelling of the basal infolds was evident after 24 hours in 0.01 µg/ml, after 12 hours in 0.1 µg/ml, after 1 hour in 1.0 µg/ml and 10.0 µg/ml. These changes become progressively more severe with longer exposure time where the cells showed disorganization and necrosis. Some of the connections between the basal infolds and the rest of the basal plasma membrane broke, so that in places the entire syncytium became detached, exposing the basal lamina beneath. These findings observed by SEM and TEM show that flukes exposed to niclosamide have all tissue damaged. This study indicates that niclosamide is an effective anthelmintic drug which can cure *H. taichui* infection. In addition, this drug has a very low side effect because it is not absorbed by the intestine and is voided with feces.