CHAPTER 2

LITERATURE REVIEW

The trematode in the genus *Haplorchis* is a group of minute intestinal flukes belonging to the family Heterophyidae. The classification of this species is based on Yamaguti, (1958); Pearson (1964); Pearson and Ow-Yang (1982).

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Heterophyidae

Subfamily Haplorchinae

Genus *Haplorchis* Loss, 1899

Species *Haplorchis taichui* Witenberg, 1930

Haplorchis taichui was described by Nishigori in 1924 under the name of *Monorchotrema taichui* based on the worms collected from dog, cat and rat in Taiwan. *Monorchotrema* was declared as a synonym of *Haplorchis* Loos, 1899 by Witenberg in 1930. *H. taichui* is one of the intestinal trematodes of the subfamily Haplorchinae. The subfamily Haplorchinae is characterized by having a single large testis and the genital sucker is partly fused with the ventral sucker and surrounded by a half circlet of genital hooklets. The body is elongate in shape and the almost entire body is covered with small spines (Figure 1). The ventrogenital sac is median, armed with 12-18 hollow spines, and arranged in a fan shape (Figure 2). The testis is single, and situated on the midline posterior to the ovary which is situated behind the ventrogenital sac

(Witenberg, 1929; Chen, 1936; Martin, 1958; Yamaguti, 1958; Pearson, 1964; Manning *et al*., 1971; Pearson and Ow-Yang, 1982).

Epidemiology

Five species in the genus *Haplorchis* have been reported in man, *viz*. *H. pleurolophocerca*, *H. pumilio*, *H. taichui*, *H. vanissimus*, and *H. yokokawai* (WHO, 1995; Waikagul 1991). Three species of *Haplorchis*, *viz*. *H. pumilio*, *H. taichui*, and *H. yokokawai* have been reported in Thailand (Waikagul, 1985; 1991; WHO, 1995). *H. taichui* infections have spread widely throughout many areas in the world, especially Southeast Asia. Adult worms are found in the small intestines of birds and mammals (Faust and Nishigori, 1926; Yamaguti, 1958; Cheng, 1964; Belding, 1965). *H. taichui* was first discovered from dog, cat and rat by Nishigori in 1924. Witenberg (1929) reported the occurrence of this species in dogs and cats in Palestine. Eggs of *H. taichui* were also recorded in fecal samples of humans, obtained Egypt and East Pakistan (= Bangladesh) (Kuntz *et al*., 1958; Kuntz, 1960). *H. taichui* was recovered from the duodenum of domestic pig (*Sus scrofa domesticus*) in India (Varghese *et al*., 1971). In Laos, larval stages of *H. taichui* were recorded in the snail, *Tarebia granifera* (Ditrich *et al.,* 1990). The infective stage (metacercaria) was found in cyprinoid fish and adults in domestic cats from Nam Ngum water reservoir. Adults of other species such as *Opisthorchis viverrini*, *H. pumilio*, *H. yogogawai* and *Stellantchasmus falcatus* were also found in domestic cats in Laos (Ditrich *et al.,* 1990). Adults of *H. taichui* were obtained from cats and eggs were also collected from human stools in Laos (Giboda *et al*., 1991; Chai *et al*., 2005). *H. taichui* was also expelled from humans in the south Philippines with 36.0 % of infections (Belizario *et al*., 2004).

In Thailand there have been reports from several investigations. *H. taichui* was first discovered from a human autopsy at Udornthani Provincial Hospital, north-eastern Thailand (Manning *et al*., 1971). *H. taichui* was later detected from naturally and experimentally infected cats, fish, and one case from ileum necropsy sections from a patient in Chiang Mai, northern Thailand (Kliks and Tantachamrun, 1974). The adult *H. taichui* expelled in human fecal samples after chemotherapy were collected in Chiang Mai and Chiang Rai Provinces (Waikagul *et al*., 1997). *H. taichui* was also recovered from humans in Phrae Province, northern Thailand (Pungpak *et al*., 1998). In freshwater fish, the cyprinoid is common intermediate host of *H. taichui* metacercariae. Seven species of cyprinoid fish collected in Chiang Mai had *H. taichui* metacercariae which were more common than *Opisthorchis viverrini* metacercariae (Sukontason *et al*., 1999). The distribution of metacercariae in the fish body of *H. taichui* metacercariae was determined in five species of cyprinoid fish. The caudal fin had the highest infection (43.39%), with the anal fin having the fewest (5.93%) (Sukontason *et al*., 2001a). However, *Haplorchis* metacercariae can be infected together with *Haplorchoides* and found mostly in the scales of cyprinoid fish in Chiang Mai Province, northern Thailand (Namue *et al*., 1998; Wongsawad *et al*., 2000). *H. taichui* was reported to be more predominant than other heterophyid species in many areas from north and north-east Thailand (Srisawangwong *et al*., 1997b; Radomyos *et al*., 1998; Sukontason *et al*., 1999). In Chom Thong district, Chiang Mai Province, 25.88% of people samples were infected with *O. viverrini* with no *H. taichui* infections (Chiang Mai Provincial Public Health office, 2001). This area also had *H. taichui* eggs in human stool while no *O. viverrini* eggs were found in the same samples (Chuboon and Wongsawad, 2003). Recently, 3 cases of *H. taichui* infection in man were reported from Lampang Province, northern Thailand (Sukontason *et al*., 2005).

Life History

The general life cycle of this fluke is shown in Figure 3. The life cycle of Heterophyid flukes is parallel to *Opisthorchis* spp., which have different intermediate hosts. The snail intermediate host of *Opisthorchis* spp. is usually *Bithynia* spp. while heterophyid flukes always infect *Tarebia granifera* and/or *Melanoides tuberculata* (Martin, 1958; Cheng, 1964; Kliks and Tantachamrun, 1974; Waikagul *et al*., 1990; Sun, 1999; Kaewkes, 1999). The operculated egg contains a fully developed miracidium. Embryonated eggs are discharged into the host's feces and hatch when ingested by a fresh water snail. The miracidium hatches in the intestines of the snail intermediate host and develops into the sporocyst, rediae, and cercaria stages in the snail's tissue. The parasite passes through a sporocyst and two generations of rediae before the cercarial stage develops. Many cercariae are produced by each redia. The cercariae are shed from the snail and penetrated into freshwater fish. They encyst and develop into an infective stage (metacercariae). After ingesting undercooked, infected fish, the metacercariae escape and mature in the small intestine of the final host including birds and mammals (Belding, 1965; Bruyning, 1985; Martin, 1958; Kaewkes, 1999; Sun, 1999).

There are many reports on the hosts of *H. taichui*. The snail intermediate hosts are *Melania obliquegranosa* (= *Tarebia granifera*) (Faust and Nishigori, 1926), *Melanoides tuberculata* (Kliks and Tantachamrun, 1974; Pearson and Ow-Yang, 1982; Farahnak and Massoud, 1999), *Thiara juncae* (Velasquez, 1973a) and *Tarebia* *granifera* (Martin, 1958; Giboda *et al*., 1991). The second intermediate hosts are freshwater fish such as *Cyprinus carpio*, *Carassius auratus*, *Zacco platypus*, *Pseudorasbora parva*, *Rhodeus ocellatus*, *Gambosia affinis*, *Ctenopharyngodon idellus* (Faust and Nishigori, 1926); *Varicorhinus* sp., *Tilapia simonies*, *Barbus canis*, *Gambosia affinis* (Witenberg, 1929), *Ophicephalus striatus* (Vazquez-Colet and Africa, 1968; Velasquez, 1973), *Oryzia latipes*, *Macropodus opercularis* (Martin, 1958) and several species of cyprinoid fish (Kliks and Tantachamrun 1974; Srisawangwong *et al*., 1997b; Sukontason *et al*., 1999; Wongsawad *et al*., 2000). The definitive hosts are some mammals *e.g.* cats and dogs (Nishigori, 1924; Faust and Nishigori, 1926; Witenberg, 1929; Martin, 1958; Velasquez, 1973a); fox, *Vulpes vulpes* (Kuntz and Chandler, 1956); and man (Faust and Nishigori, 1926; Africa *et al*., 1935; 1936; 1937a; 1937b; Manning *et al*., 1971; Kliks and Tantachamrun, 1974; Waigakul, 1991; Radomyos *et al*., 1994; 1998; Pungpak *et al*., 1998; Chuboon and Wongsawad, 2003). In addition, some bird and fowl can be served as the definitive host of fluke such as cattle egrets (*Larus* sp.), ducklings (Witenberg, 1929) and chicks (Velasquez, 1973a).

The life cycle of *H. taichui* was reported for the first time from Formosa, Taiwan. The first intermediate host of *H. taichui* is a freswater snail (*T. granifera*). The miracidium penetrates the snail's tissues and after five to six weeks mature lophocercous cercariae develop in the rediae. Maturation of metacercariae can be observed from 13-15 days after the cercariae infect the fish. On the seventh or ninth day after infection in dogs and cats the flukes become fully mature (Faust and Nishigori, 1926). Martin (1958) reported the life history of *H. taichui* in Hawaii. Parapleurolophocercous cercariae were collected from the naturally infected snail (*Tarebia granifera*). Experimental infections were established in fish *viz*. *Gambusia* *affinis*, *Mollienesia formosanus*, and *Carassius auratus*. Adult worms were recovered from the small intestine of cats. In Iran, the life cycle of *H. taichui* was studied under laboratory conditions. The fluke's eggs were exposed to *Melanoides tuberculata* or *Melanopsis* spp. and cercariae develoed, the second intermediate freshwater fish (*Gambosia affinis*) was infected with cercariae and then metacercariae were obtained after one month. Adults were recovered from the infected domestic cats, rats, chickens and mouse (Farahnak and Massoud, 1999).

In Thailand, there is little knowledge of the life history on some species of heterophyid flukes. *H. yokogawai* was collected from a naturally infected snail (*Melaniodes tuberculata*), cyprinoid fishes and cat from Pakplee District, Nakornnayok Province, Central Thailand. *Cyprinus carpio* and albino rats were used as the experimental host of this fluke (Vajrasthira *et al*., 1971). *Centrocestus caninus* was recovered from a naturally infected snail, *Melaniodes tuberculata*, collected from Nakornnayok and Saraburi Provinces. In the laboratory, metacercariae were found to encyst in gill rays and under the scales of *Cyprinus carpio* and *Tilapia nilotica*. Adult flukes were recovered from the experimental white rat (Waikagul *et al*., 1990). In Chiang Mai Province experimental hosts have been found in some species. There is a report on the experimental definitive host for *Stellantchasmus falcatus* that includes mice and chicks. Flukes were distributed in the small intestine of chicks and mice with 70% and 80% of incidence and intensity of infection ranging from 1-44 (24.6) in chicks and 1-67 (7.8) in mice after forced fed with 200 metacercariae (Wongsawad *et al*., 1998). In *H. taichui*, parapleurolophocercous cercariae were shed by *Melanoides tuberculata* from natural infection. These cercariae were experimentally exposed to the cyprinoid fish, *Puntius gonionotus*, and then developed into *H. taichui* and

H. yokogawai metacercariae at 5 days post-infection (Kliks and Tantachamrun, 1974). The development of *H. taichui* was studied in mice. Sukontason *et al*. (2001b) reported that two peaks of increment in the length and width of worms were found at 3 and 7 day post-infection with the rapid maturation of worms during 3 days post-infection.

Tegumental Surface Study

Surface structure of heterophyid flukes has been studied to understand their taxonomic, morphological, and functional characteristics. The surface structure of *Haplorchis pumilio*, the adults were collected from experimentally infected hamsters. The surface is covered with scale-like multi-pointed spines regularly arranged in transverse rows. Ciliated papillae are present on the anterior two-thirds of the body, especially on the oral sucker (Srisawangwong *et al*., 1989). *H. yokogawai* adults were obtained from naturally infected cats, while *H. taichui* adults were obtained from cats and humans. *H. yokogawai,* adult surface covered with multi-pointed spines provided with 5-16 points. Adult surface of *H. taichui* covered with multi-pointed spines contained 5-12 points (Scholz *et al*., 1991). *Heterophyes nocens* adults were recovered from experimentally infected cats and a naturally infected human patient. The tegumental surface is covered with multi-pointed spines with the number of tip points decreasing posteriorly. The anterior part has 5-9 points, between the oral sucker and ventral sucker have 12-17 points, then decreased to 1-3 points. Ciliated knob-like papillae distributed singly or in groups and non-ciliated round swellings are present on the lip of the ventral sucker (Chai *et al*., 1992). Adults of *Stellantchasmus* sp. were collected from the rat, *Rattus norvegicus*. Almost the entire surface is covered with scale-like spines, each with 7-9 points. The papillae consist of a single club-like cilium.

Single or groups of papillae are more common on the anterior part of the body than on the other parts (Wongsawad *et al.*, 1997). The adult of *Stictodora fuscatum* was recovered from a kitten fed with infected mullets. The surface of the worm is covered with scale-like, multi-pointed spines digitated into 9-12 points in the anterior region and posteriorly decreasing in size and number of tip points. Ciliated sensory papillae, single or in groups, are present on the rim of the oral sucker and anterolateral surface, while non-ciliated papillae appear on the lower lip of the oral sucker (Abdul-Salam *et al.*, 2000). *Acanthotrema felis*, the adults were recovered from a kitten experimentally infected with metacercaria. The outer surface is covered with scale-like multi-pointed spines. The anterior spines have 10-12 pointed tips, while posterior ones have 6-8 pointed tips. Obviously, no sensory papillae were observed on the tegumental surface (Sohn *et al.*, 2003).

The scanning electron microscope has been used to study the surface ultrastructure of *H. taichui* adults, which is covered with scale-like, multi-pointed spines. The number of spines from the anterior region has 5-7 points which become slightly larger and with 9-12 points. Behind the ventrogenital sac the tegument is covered with 7-8 pointed spines and the number of points decreases posteriorly (Scholz *et al.*, 1991). The metacercariae of *H. taichui* have 3-11 spine tips. The anterior spines are digitated into 10-11 points, then 8-9 points, 7 points, and 3 points posteriorly. Two types of sensory papillae disposed on the body surface. Type I is a ciliated knob-like swellings and type II is a round swellings of the tegument (Sukontason *et al.*, 2000a). Studies on the surface ultrastructure of metacercariae and adults of Heterophyidae have been done for some species. The surface ultrasructure of metacercariae and adults of *Heterophyopsis continua* were observed by SEM.

The tegumental processes are band-like in the metacercariae, cobblestone-like in 2 days post-infection, and velvety at 3 days post-infection. Tegumental spines between the oral and ventral suckers are digitated with 10-14 points while in 6 days post-infection flukes the number of points is 15-17 (Hong *et al.*, 1991). Metacercariae of *Centrocestus formosanus* isolated from *Puntius* spp. and adults collected from infected hamsters. The oral sucker is surrounded by a circumoral expansions with two rows of 32 spines. The surface is covered with scale-like, multi-pointed spine. At 4 weeks, the oral and circumoral expansions are cobblestone-like and the spine grooves become split (Srisawangwong *et al.*, 1997a). In excysted metacercariae of *Metagonimus takahashii*, tegumental spines are dense and digitated into 5-7 points on the surface anterior to the ventral sucker, but become sparse and less digitated posteriorly. In adults, tegumental spines on the anterior two-thirds of the body are digitated with 9-12 tips on the ventral and 8-13 tips on the dorsal surface (Chai *et al.*, 2000).

Surface ultrastructural studies with a scanning electron microscope can be distinguished species and improve knowledge to aid in the control and protection of worm infections, especially with the usage of the anthelmintic drugs. *H. taichui* showed that the spines on the tegument were damaged after treatment with praziquantel (Scholz *et al.*, 1991). SEM observations have shown the differentiation of the egg shell of some heterophyid flukes and the liver fluke (*Opisthorchis viverrini*) which are always mixed in the same stool samples. *O. viverrini* eggs look like having musk-melon skin with prominent shoulders and long knobs. *H. taichui* eggs have curly, thread-like ridges and prominent shoulders and knobs. *H. pumilio* eggs have elongated ridges, like Chinese bitter mormodica, and prominent shoulders. These findings are very difficult to observe under a light microscope (Tesana *et al.*, 1991).

Pathogenicity

Digenetic trematodes in the intestine rarely seem to be pathogenic and cause a mild inflammatory response at the site of attachment of the oral and ventral suckers. The clinical situation of trematode infections depends on three conditions: size and number of worms present in the host, organs or tissues parasitized, and the degree to which parasites including eggs, exist in the tissues rather than in the intestinal lumen or in the cavities of the host (Beaver *et al.*, 1984). There are some document reports for the pathogenicity of *H. taichui* in the small intestine of man from Thailand. Three cases heavily infections with this worm presented mucosal ulceration, mucosal and submucosal haemorrhages, fusion and blunting villi, crypt hyperplasia, chronic inflammation and fibrosis of the submucosa (Sukontason *et al.*, 2005). Moreover, there have been reported on other heterophyid flukes. In heavy infections the worms cause abdominal pain, mucous diarrhea, ulceration of the intestinal wall and superficial necrosis of the mucous coat (Beaver *et al.*, 1984; Garcia, 2001; Chai and Lee, 2002; Pica *et al.*, 2003). The intestinal histopathology caused by heterophyid flukes has been studied in *Metagonimus yokogawai* and *Stellantchasmus falcatus*. The pathological features are characterized by villous atrophy and crypt hyperplasia with variable degrees of inflammatory reactions. The infected mucosa shows blunting and fusion of the villi, edema of the villus tips, congestion and inflammatory cell infiltrations in the villous stroma, and decreased villus/crypt height ratios (Chai, 1979; Chai *et al.*, 1990; Tantachamrun and Kliks, 1978). Some eggs of this fluke will escape though the tissues of the other appropriate organs (Africa *et al.*, 1937a; 1937b). Fluke's egg drift to deeper viscera also occurs in submucosal tissues. In this way eggs can be trapped in the submucosal tissues, lymphatics, mesenteric venules or peripheral blood vessels which can become deposited in the spinal cord or heart of man (Neva and Brown, 1994). Such cases have been reported in other *Haplorchis* species in the Philippines. Africa *et al*. (1935, 1937a) found eggs of *H. taichui* in cardiac lesions of persons dying of cardiac failure and in one case adult heterophyids were found in the epicardial layer of the heart (Africa *et al*. 1937b).

Treatment and chemotherapy

For treatment of adult *H. taichui* infections, the drug of choice is normally praziquantel (Pungpak *et al*., 1998; Radomyos *et al*., 1998; Chai *et al*., 2000; 2005). Niclosamide and albendazole have also been recommended for treatment of this worm at a dose of 40 mg/kg body weight (Sukontason *et al*., 2000b; Waikagul *et al*., 2003). Niclosamide is effective in treatment of many kinds of trematode and cestode infections. This drug inhibits oxidative phosphorylation in the mitochondria of cestodes and has low side effects because it is not absorbed from the gastrointestinal tract (Katz, 1986). There may be some side effects by this drug, including nausea, vomiting, diarrhea, dizziness and abdominal pain (Suntharasamai *et al*., 1974; Garcia, 2001). However, side effects due to niclosamide are mild when compared with other drugs. This medicine also inhibits the uptake of glucose by the helminth and stops its production of energy. It has a paralytic effect on the worm. The drug works by killing worms, and the killed worms pass in the stool. However, sometimes the worm cannot be collected after treatment because it can be destroyed in the host's intestine (Rat Medication Guide, 2003). Niclosamide at a dosage of 6 g over 3 alternate days has been recommended for *Heterophyes heterophyes* infection (Khalil and Rifaat, 1964) and 100-125 mg/kg for *Metagonimus yokokawai* infection (Rim, 1975).

The advantage of niclosamide 40 mg/kg has been demonstrated with *H. taichui* in mice (*Mus musculus*), a 100% cure rate was found (Sukontason *et al*., 2000b). However, there are no reports about the effects of this drug on the tegumental surface of *H. taichui*. It has been reported that niclosamide treatment is effective in *Gyrodactylus aculeati* and *Diplozoon paradoxum* parasitizing the skin and gills of fish. The drug was effective against *Gyrodactylus aculeati* in a narrow concentration range of 0.075-0.1 μg/ml. A dose of 0.2 μg/ml of niclosamide was effective against *Diplozoon paradoxum*. Niclosamide caused damage of the parasite tegument (Schmahl and Taraschewski, 1987).

Little is known on the effect of drugs on *H. taichui*. The effect of praziquantel on *H. taichui* showed partly damaged tegument and the widened basal part of spines after an *in vivo* treatment (Scholz *et al*., 1991). Studies have been conducted on the effects of praziquantel on some other species of trematodes. *Fibricola seoulensis*, an intestinal fluke, was exposed to 0.01-100 μg/ml of praziquantel for 24 hours. The lowest effective lethal concentration was 0.1 μg/ml. Severe tegumental changes consisted of bleb formation followed by rupture and loss of the tegumental surface (Lee, 1985). *Paragonimus westermani*, a lung fluke, was observed after *in vitro* incubation in various concentrations of praziquantel solution. Flukes were immobilized immediately after incubation in 0.1 μg/ml. Surface damage included bleb formation and then ruptured with destruction of the syncytium and large craters appearing. The treated worms showed severe vacuolization in the tegument, intestine, ovary, testis, Mehlis' gland, and excretory bladder (Lee *et al*., 1987). *Opisthorchis viverrini*, a liver fluke, was investigated *in vitro* incubation in 0.1, 1.0 and 10.0 μg/ml praziquantel for 60 minutes. The surface damage consisted of blebbing due to the swelling of microvilli, followed by the disruption of blebs to form lesions on the surface. The ventral and dorsal surfaces exhibited similar changes, while the anterior part was less damaged than the posterior part. In TEM observations, the microtrabecular network was disrupted causing the formation of non-membrane-bound vacuoles (Apinhasmit and Sobhon, 1996). *Schistosoma mansoni*, a blood fluke, was treated *in vivo* with praziquantel. The damage induced by praziquantel on the tegument after 1 hour included numerous blebs forming on the surface and some blebs rupturing to form lesions (Liang *et al.*, 2002). Moreover, *Fasciola hepatica*, a liver fluke, has been treated in many investigations. The effect of the deacetylated (amine) metabolite of diamphenethide (10 μg/ml) on the tegument of *F. hepatica* over 24 hours *in vitro* was observed by TEM. T2 secretory bodies accumulated at the apical surface after 6 hours with increased exocytocysis of secretory bodies and blebbing of the surface membrane (Fairweather *et al.*, 1986). In addition, *F. hepatica* was treated with the deacetylated (amine) metabolite of diamphenethide (10 μg/ml) for 24 hours. Blebbing occurred around the oral sucker after 3 hours and then moving posteriorly by 12 hours *in vitro*. The blebs increase in size and burst, causing lesions and loss of spines (Fairweater *et al.*, 1987). *F. hepatica* were also examined after *in vivo* treatment with clorsulon at a concentration of 12.5 mg/kg body weight. After 24 hours, disruption of the tegumental syncytium was noticeable while some blebbing at the apex was also present and the mitochondria were slightly swollen. After 48 hours, more severe blebbing and vacuoles appeared in the syncytium. The gut showed damage after treatment with disrupting of the surface lamellae and appearance of autophagic vacuoles at the apex of the cells. The mitochondria and the granular endoplasmic reticulum became swollen and lipid droplets were present in the cells (Meaney *et al.*, 2004).

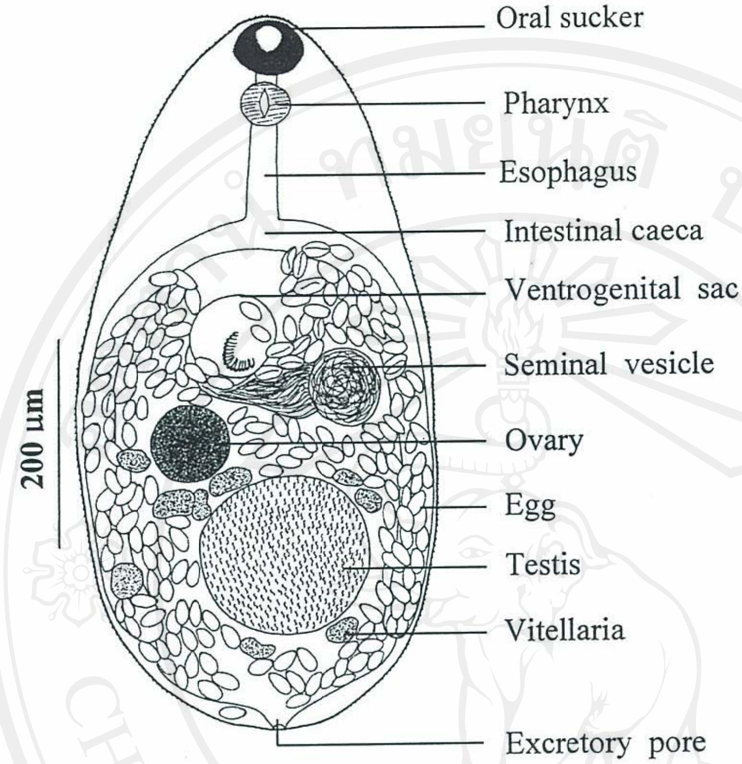

Figure 1. Adult of *Haplochis taichui* from experimentally infected chick 3 days post-infection.

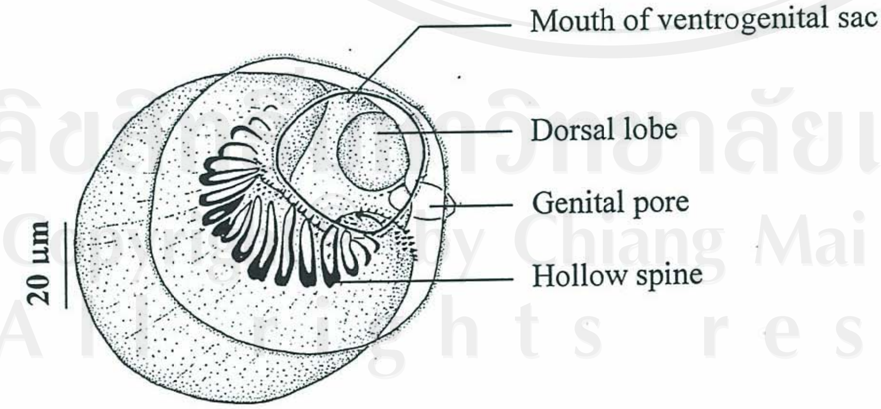

Figure 2. The ventrogenital sac of *Haplochis taichui* with hollow spines in a fan-shaped. (Modifield from Pearson and Ow-Yang, 1982)

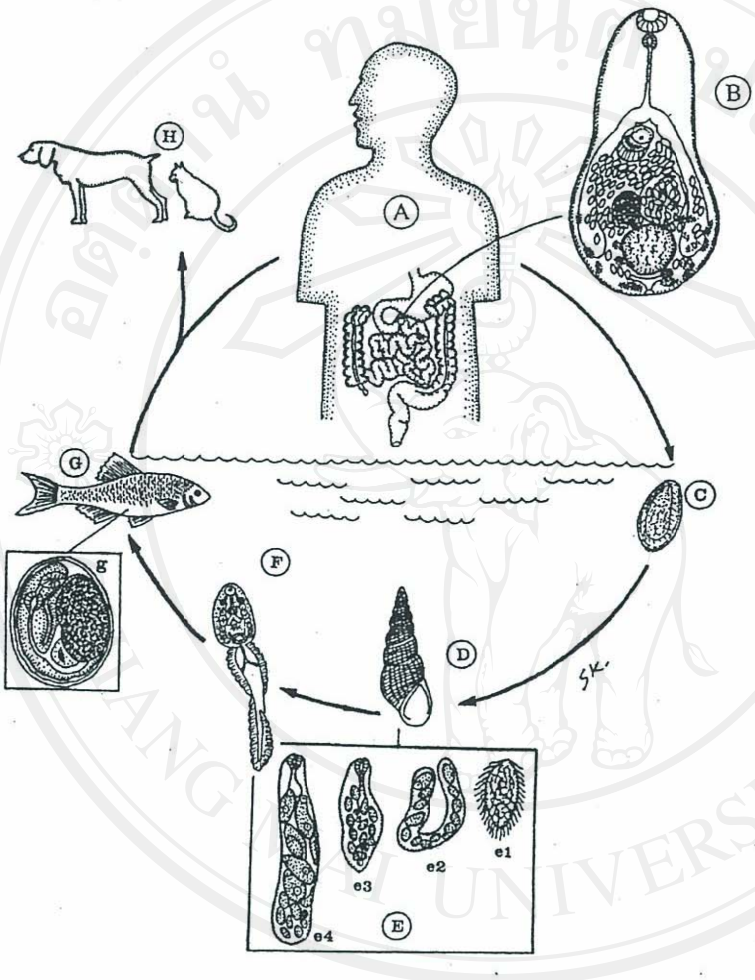

Figure 3. Life cycle of *Haplorchis taichui*. A : definitive host, human; B : Adult in small intestine; C: embryonated egg; D : first intermediate host, *Melanoides* sp; E : intramolluscan stages, miracidium (e1), sporocyst (e2), mother rediae (e3), daughter rediae (e4); F : cercaria; G : second intermediate host (cyprinoid fish), metacercaria in fish (g); H : reservoir host, dog and cat (Kaewkes, 1999).