Original Article

Prevalence and Histopathology of *Trichogaster pectoralis* Harbouring Metacercaria of *Clinostomum piscidium* (Southwell and Prashad, 1918) in Central Thailand

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Abstract

The aim of this study was to identify the digenea in abdominal cavity of *Trichogaster pectoralis* from two fish farms in Ban Paew District, Samutsakorn Province, Central Thailand, and to investigate the prevalence, intensity and pathological alterations caused by fluke in the host fish. Two hundred and seventy three 8 to 10- month-old *T. pectoralis* were obtained from two farms (60 females and 75 males from the first and 63 females and 75 males from the second). Their total length and body weight were measured. Metacercariae of *Clinostomum piscidium* were identified in the body cavity of *T. pectoralis*. The parasites were found either free or attached to adipose tissue and external surface of visceral in abdominal cavity of the infected fish. The prevalence and parasite intensities were greater in the females than in the males collected from both farms (p<0.01). The infected fish appeared starved with significantly decreased body weight when compared to the uninfected fish (p<0.01). Gross pathological findings revealed a few white migratory tracks on hepatic surface. Histologically, the track appeared as central hepatic and pancreatic cells necrosis and hemorrhage surrounded by a layer of macrophages and epithelioid cells, surrounded by a rim of lymphocytes, eosinophilic granular cells and fibroblasts. Eosinophils were in close contact with the fluke's tegument. The infection with this metacercaria caused hepatic tissue damage which, in turn, interrupted the hepatic metabolism, causing growth retardation and a decrease in body weight of the fish hosts.

Keywords: *Clinostomum piscidium*, histopathology, metacercaria, prevalence, *Trichogaster pectoralis*

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Introduction

Trichogaster pectoralis or snakeskin gourami is a teleost fish known to Thai people as "Pla-salid". In Thailand, Pla-salid fish farms are plentiful in Nakornpathom, Samutprakarn, and Samutsakorn Provinces, which are the main sources of supply to the market. In 2011, The Department of Fisheries reported the income from exporting the products of Gourami fish at 1,756.3 million bahts (Department of Fisheries, 2011). Infection by parasites *Clinostomum piscidium* constitutes one of the most significant problems associated with pond fish culture, including decrease in weight, reduction in growth, and loss of production (Paperna, 1991). Due to this parasitism, the fish become susceptible to other health problems which can lead to death. *C. piscidium* is a trematode parasite belonging to the family Clinostomidae, which has a complex life cycle and needs at least two intermediate hosts for completing the whole cycle (Leidy, 1856; Singh, 1959). The first intermediate host is the snail, *Lymnaea lutiola*, and the serpent-head fish, *Ophiscephalus punctatus* is the second intermediate host, in which the metacercariae move actively in its body cavity. The final host are fish eating birds, *Bulbulcus ibis* and *Egretta grazetta*, where adult flukes grow up in their buccal cavity 5-6 days after being ingested (Singh, 1959). Metacercaria of *C. piscidium* was first reported in India through its recovery from the body cavity of the fish, *Nandus nandus* (Southwell and Prashad, 1918). Later, the metacercariae were also discovered in the abdominal cavity of the same fish species (Bhalerao, 1942). Subsequently, a few more fish species have been reported to be infected by *C. piscidium*, including *Colisa fasciata* (Pandey and Baugh, 1969) and *Macrognathus aculeatus* (Khanum et al., 2011). In 1985, metacercaria of *C.piscidium* was detected in the abdominal cavity of *T. pectoralis and T. trichopterus* from Samutprakarn Province, Thailand (Charoenpornsook, 1985). In *T. pectoralis*, the prevalence was 54.42% and mean intensity of parasite was 6.48. The infected fishes showed significant reduction in body weight and length. Recently, other clinostomid parasites were also reported in the North and Central of Thailand. The metacercariae of *C. philipinensis* were detected in the gill of *T. microlepis* from Nakhonsawan province (Yooyen et al., 2006). Ngamniyom et al. (2012) reported the occurrence of encysted *C. complanatum* in the skin of *Oryzias minutillus* from Pathumthani province. Although there had been an initial unpublished report by Charoenpornsook (1985), information on histopathological alterations in fish parasitized with this *C. piscidium* metacercaria are still lacking since. The present study aimed to investigate the prevalence, intensity and pathological change in *T. pectoralis* as a result of *C. piscidium* infection, since this parasite critically affect the production of *T. pectoralis*, which is one of the major economic fish being cultured in Thailand.

Materials and Methods

Fish and parasite collection: One hundred and thirty five 8 to 10 month-old *T. pectoralis* were collected from farm No. 1 (60 females and 75 males) and 138 fish were collected from farm No. 2 (63 females and 75 males) in Ban Paew District, Samutsakorn Province, Central Thailand. The fish were cultured in earth pond and the population density was 10,000 fingerlings per rai. Water used for the fish farms was supplied from nearby irrigation canal. These two farms were approximately 25 km apart. Fish were euthanized with tricaine methansulphonate (250 mg/l). The total length and body weight of each fish were measured. Necropsy was performed and the exposed body cavity with all visceral organs intact were examined with naked eyes for parasites. Subsequently, their visceral organs were dissected and transferred to Petri dish, rinsed with 0.85% NaCl (NSS) and searched for parasites. If present, they were gently removed and placed in NSS. The number of parasites were recorded, then some samples were processed for light microscopic examination. The animal care and handling in this study was approved by Faculty of Veterinary Science – Animal Care and Use Committee, Mahidol University (FVS-ACUC Protocol No 2008-27).

Prevalence was calculated according to the number of infected fish divided by the total number of fish examined and expressed in percent. Mean intensity of parasite infection was determined by dividing the total number of recovered parasites by the number of infected fish.

Statistical analysis: Data collected from the infected and non-infected fish were compared by one way analysis of variance (ANOVA) with Duncan's post hoc. *P* value lower than 0.01 was considered as significant.

Specimen preparation for light microscopic examination: Small pieces of liver and intestine were fixed in 10% buffered formalin, dehydrated through a graded series of ethanol, embedded in paraffin, sectioned serially in 3 µm thickness and stained with hematoxylin and eosin (H&E). They were observed in a light microscope (Nikon ECLIPSE E 200 -Japan) and photographed.

For Carmine staining, the metacercariae were flattened between two glass slides. They were fixed in ethanol-formalin acetic acid (AFA) for 7 days, washed with 70% ethanol, stained by Semichon's carmine and washed in acid ethanol. Then, the specimens were dehydrated in an increasing concentrations of ethanol, cleared in xylene and mounted in permouth, and observed in a Olympus SZ 61 stereomicroscope and photographed.

Results

Identification of parasite: The parasite was yellowish-

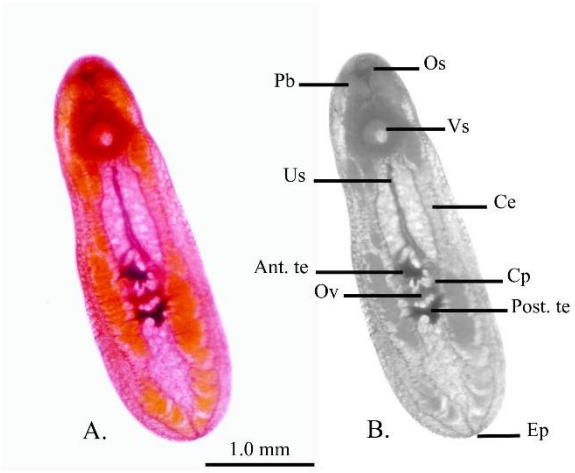

Figure 1 Light micrographs of metacercaria of *Clinostomum piscidium*. A) The whole mount (Semichon's carmine stained). B) Details of a whole mount: Oral sucker (Os), Ventral sucker (Vs), Pharyngeal bulb (Pb), Intestinal ceca (Ce), Anterior testis (Ant. te), Posterior testis (Post te), Uterine sac (Us), Ovary (Ov), Cirrus pouch (Cp), Excretory pore (Ep).

white in color and its body was linguliform and dorso-ventrally flattened with round anterior and posterior ends (Fig 1A). The total length of the fluke was 2.4-4.2 mm while the maximum width at the middle and posterior one- third of the body was about 1.2-1.7 mm. The oral sucker was at the anterior tip, slightly oval, measured 0.09-0.20 mm wide and 0.25-0.35 mm long. The ventral sucker was situated at the anterior one-third of the body, larger than the oral sucker, oval shaped and, measured 0.48–0.70 mm wide and 0.62–0.85 mm long. The pharynx was very short and dilated into the pharyngeal bulb. The intestinal caeca ran laterally along both sides of the body to the posterior end. The testes were are paired, situated in tandem along the median longitudinal axis at the posterior one-third of the body. The anterior testis was roughly triangular in shape with lobulated margins, slightly off to the left of the median axis. The posterior testis was Y-shaped and symmetrically located on the median axis. Between the two testes were the cirrus pouch and the ovary, with the oval cirrus pouch, lying anterior to the ovary (Fig 1B). The ovary had an oval shape, lying posterior to the cirrus pouch on its left. The uterine sac was long, tubular shaped with blind end, situated behind the posterior margin of ventral sucker. At the posterior end was the excretory pore, which is the opening of excretory bladder (Fig 1B).

The main morphological characters of metacercaria in this study were compared to other reports on metacercariae of *C. piscidium* as presented in Table 1.

Prevalence, intensity and susceptibility of Trichogaster pectoralis to Clinostomum piscidium: In this study 120 out of the 273 of *T. pectoralis* were infected with metacercaria of *C. piscidium*. The overall prevalence of infection was 43.95% out of which 42.96% infection was recorded in farm No. 1 and 44.93% in farm No. 2. Regarding the hosts' gender, the prevalence of infection was 50% in the females and 37.33% in the males from farm No. 1 and 52.38% in the

Table 1 Comparison of morphological characteristics of *Clinostomum piscidium* metacercariae found in the present study and described previously

Morphological characteristics	Southwell & Prashad, 1918	Chareonpornsook, unpublished data	Singh et al., 2010	Present study
Body length (mm)	2.8–5.2	2.33-8.13	2.27–3.36	2.4-4.2
Body width (mm)	1.4–1.8	0.9–2.18	1.17–1.26	1.2-1.7
Size of oral sucker (mm)	0.18 mm in diameter	0.12-0.37 x 0.2-0.67	0.14-0.18 x 0.23-0.3	0.09-0.20 x 0.25-0.35
Size of ventral sucker (mm)	0.6 x 0.48	0.35-1.03 x 0.4-1.05	0.60 -0.68 x 0.7 -0.8	0.48-0.7 x 0.62-0.85
Location of gonad	Behind the middle of the body	Posterior third of the body	Middle third of the body	Behind the middle of the body
Shape of ovary	Oval or bean	Oval or bean	Globular	Oval
Shape of ant. testis	lobed	lobed	Lobed	Triangle, lobed
Shape of post. testis	Y-shaped	Y-shaped	Y-shaped	Y-shaped
Host species	*Nandus nandus*, *T. fasciatus*, *T. pectoralis*	*T. pectoralis*, *T. trichopterus*	*Colisa fasciata*	*T. pectoralis*
Microhabitat	Body cavity	Body cavity	Body cavity	Body cavity
Metacercariae form	Not encysted	Not encysted	Not encysted	Not encysted

Table 2 Prevalence and intensities of infection with metacercariae of *Clinostomum piscidium* in male and female *Trichogaster pectoralis*

Localities	Sex of fish	No. of examined fish	No. of infected fish	Prevalence (%)	Mean intensity of parasite (mean ± SD)	Significance
Farm No. 1	Female	60	30	50.0	13.67 ± 6.87	$p<0.00$
	Male	75	28	37.33	7.07 ± 13.13	
Farm No. 2	Female	63	33	52.38	14.45 ± 5.89	$p<0.00$
	Male	75	29	38.67	4.79 ± 4.93	

Table 3 Relationship between body weight and total length of *Trichogaster pectolaris* infected with *Clinostomum piscidium*

Locality	Sex	Status of infection	No. of fish	Body weight (mean ± S.D.,gm)	Significance	Total length (mean ± S.D., cm)	Significance
Farm No. 1	Female	Non-infected	30	159.87 ± 21.92	$p<0.01$	20.43 ± 1.10	$p>0.05$
		Infected	30	94.33 ± 31.70		17.60 ± 2.18	
	Male	Non-infected	47	123.62 ± 27.54	$p<0.01$	19.36 ± 1.72	$p>0.05$
		Infected	28	80.36 ± 38.49		16.50 ± 2.89	
Farm No. 2	Female	Non-infected	30	156.67 ± 20.54	$p<0.01$	19.63 ± 1.10	$p>0.05$
		Infected	33	92.24 ± 29.92		17.52 ± 2.09	
	Male	Non-infected	46	125.70 ± 26.84	$p<0.01$	19.43 ± 1.63	$p>0.05$
		Infected	29	86.90 ± 34.40		18.49 ± 2.40	

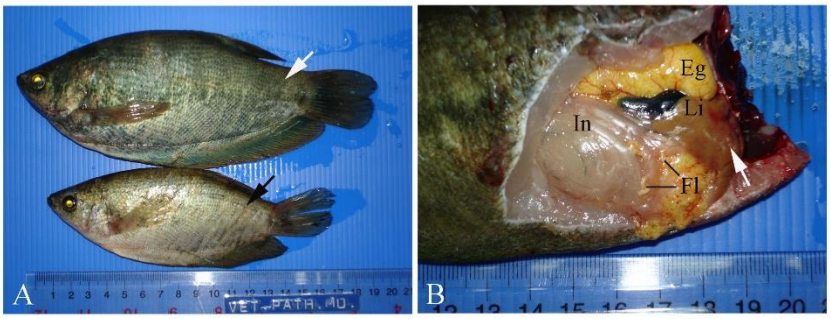

Figure 2 Micrographs of *Trichogaster pectoralis*. A) Comparison between non-infected fish (white arrow) and infected fish (black arrow) in the same pond B) Abdominal cavity of infected fish shows metacercariae of *C. piscidium* (Fl) on the serosal surface of the intestine (In). Numerous raised white tracks (arrows) are present in the liver (Li)

females and 38.67% in the males farm No. 2 (Table 2). The intensities of infection were 13.67 ± 6.87 in the females and 7.07 ± 13.13 in the males of *T. pectoralis* collected from farm No. 1. In farm No. 2, the intensities were 14.45 ± 5.89 and 4.79 ± 4.93 in the females and males respectively. The percentage of infection and the intensity were significantly higher in the female fish than the male collected from both farms ($p<0.01$, Table 2). The mean of body weight in the females and males is significantly different between the infected and non-infected fish from both farms, which were 94.33 ± 31.70 gm and 159.87 ± 21.92 gm in the females ($p<0.01$) and 80.36 ± 38.49 gm and 123.62 ± 27.54 gm in the males ($p<0.01$) from farm No. 1, respectively. Similarly, the body weight of the infected and non-infected fish from farm No. 2 were 92.24 ±29.92 gm and 156.67 ± 20.54 gm in the females($p<0.01$) and 86.90 ± 34.40 gm and 125.70 ± 26.84 gm in the males ($p<0.01$), respectively (Table 3). The body length of infected and non-infected female and male fish collected from farm No. 1 were 17.60 ± 2.18 cm and 20.43 ± 1.10 cm ($p>0.05$) and 16.50 ± 2.89 gm and 19.36 ± 1.72 cm ($p>0.05$), respectively. In farm No. 2, the body length of infected and non-infected females and males of *T. pectoralis* were 17.52 ± 2.09 cm and 19.63 ± 1.10 cm ($p>0.05$) and 18.49 ± 2.40 g and 19.43 ± 1.63 cm ($p>0.05$), respectively. There is no significant difference among the body length values of fish from both farms (Table 3).

Pathology: The infected fish appeared starved with reduction in size when compared to the uninfected fish in the same pond (Fig 2A). Gross pathological findings were marked on the liver. The liver was slightly yellowish in color with a few greyish-white irregular lines measuring1-2 mm x 3-4 mm appearing on the external and cut surface (Fig 2B). Metacercariae of *C. piscidium* were found as non-encysted form, either freely moving or attaching to the adipose tissue and external surface of the visceral organs in the abdominal cavity of the infected fish (Fig 2B). Histologically, the liver of the non-infected fish showed no significant pathological change and revealed hepatocytes arranged in anastomosing cords around a central vein (Fig 3 A, B). The hepatopancreas had an acinar arrangement and lay around a branch of portal vein (Fig 3 A, C). The liver of *T. pectoralis* harbouring metacercariae of *C. piscidium* was marked by the presence of migratory track in the liver parenchyma (Fig 3D). The track was characterized by cavitation which was filled with massive hepatic and pancreatic tissue necrosis with hemorrhage and surrounded by consecutive layers of macrophages and epithelioid cells, and a peripheral rim of lymphocytes, eosinophilic granular cells and fibroblasts (Fig 3E,G). The metacercariae of *C. piscidium* adhered to the serosal surface of the intestine and were adjacent to the migratory track (Fig 3D,F). A number of eosinophilic granular cells and macrophages were observed either in the space between the host necrotic tissue and the fluke or in close contact to the parasite tegument (Fig 3H).

Discussion

In the present investigation, the parasites recovered from the body cavity of *Trichogastor pectoralis* showed morphological characteristics which were similar to the metacercaria of *Clinostomum piscidium* previously described by Southwell and Prashad (1918), Charoenpornsook (1985), and Singh et al. (2010). The

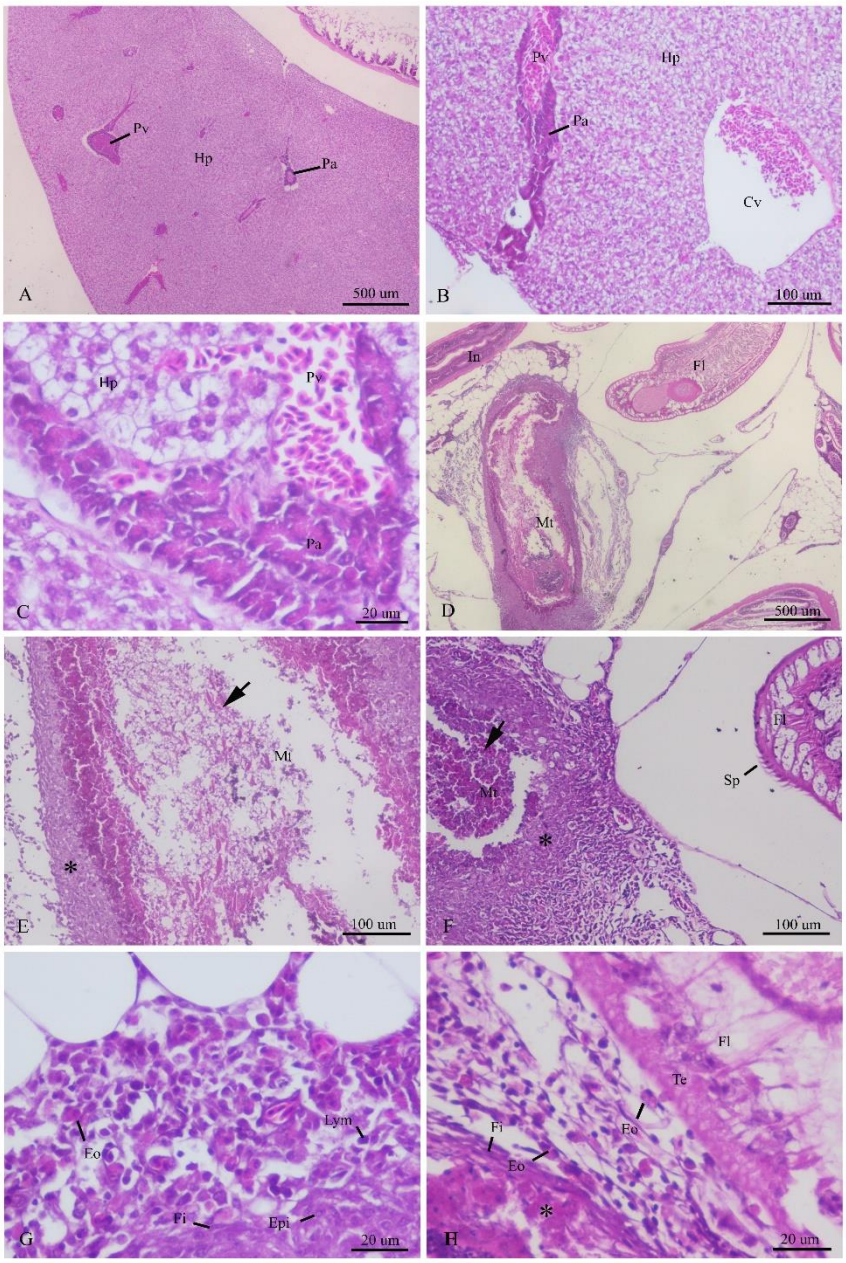

Figure 3 Light micrographs of visceral organs of *T. pectoralis* non-infected. (A-C) and infected (D-H) with *C. piscidium* (H&E stained). A) Liver of non-infected fish shows normal arrangement of hepatocytes (Hp) into cords or plates. Hepatopancrease (Pa) lies around a portal vein (Pv). B, C) Higher magnification of Fig 3A. shows hepatocytes (Hp) lying around central vein (Cv), hepatopancreas (Pa), portal vein (Pv). D) Liver of infected fish shows migratory track (Mt), metacercaria of *C. piscidium* (Fl) present as a non-cysted form near the track, intestine (In). E, F) Higher magnification of the migratory track in Fig 3D. shows area of massive hepatic necrosis (arrow) in the center and a layer of macrophages, epithelioid cells and fibroblasts at the periphery (asterisk). Metacercaria of *C. piscidium* shows tegumental spine (Sp). G) Higher magnification of the outer layer of the track in Fig 3F. shows a mixture of inflammatory cells which includes eosinophilic granular cells (Eo), lymphocytes (Lym), epithelioid cells (Epi) and fibroblast (Fi). H) A number of fibroblast (Fi) and eosinophilic granular cells (Eo) are observed at the outer layer of the track (asterisk) in close contact with parasite's tegument (Te); Fluke (Fl)

position of gonad at the posterior one-third of the body and the y-shaped posterior testis are the unique characteristic in this species. The metacercariae of *C. piscidium* were described for the first time in *Nandus nandus* in India (Southwell and Prashad, 1918). In Thailand, Charoenpornsook (1985) reported the finding of this metacercariae from the body cavity of *T. pectoralis* and *T. micropterus*. In the present study, the metacercariae of *C. piscidium* were recovered from only the abdominal cavity of the infected fish. Similar findings were also found in *N. nandus* (Southwell and Prashad, 1918), *Colisa fasciata* (Singh et al., 2010), and *M. aculeatus* (Khanum et al., 2011). These indicate the highly specific microhabitat of the parasite in their hosts. In the present study, the overall prevalence of *C. piscidium* infection in *T. pectoralis* was rather high (43.95 %) and nearly similar in fish collected from both farms. It was revealed that there was a relationship between hosts' sex and parasite infection and intensity, as the female fish was more susceptible to the infection than the male fish. The rate of infection and worm intensity were greater in the females than in the males from both

farms. Records on greater infectivity in female hosts is also reported by Kalantan (1987), who observed that the prevalence of *C. complanatum* in *Aphanius dispar* was higher in females as compared to males.

The prevalence and intensity of endoparasites infection in female of *Channa punctatus* were also greater than males (Alam et al., 2010). Gholami *et al*, (2011) also reported the greater prevalence of *C. complanatum* infection in female and male *A. dispar* at 1.33% and 1%, respectively. On the contrary, there have also been records on higher infectivities in males as compared to females. Ochieng et al., (2012) reported the greater *C. tilapia* infection in male of *Oreochromis niloticus* than the female fish. Factors contributing to variations in parasite infectivity in different sexes are unknown. Higher prevalence and intensity of parasite infection in female hosts could be due to the physiological and biological factors that make females more susceptible and allow parasites to survive better. On the other hand, the male fish may be stronger to resist the infection and allow lesser degree of parasite development (Siddiqui and Nizami, 1982). The differences in degree of infection between genders may also be related to behavioral difference between males and females (Thompson and Kavaliers, 1994). It has been known that there are two forms of metacercariae of Clinostomids inside the host tissue, which are encysted and non-encysted forms. *C. piscidium* is the only species whose metacercaria resides in the cavity of the second intermediate host without encystment. The non-cysted metacercaria of this species may cause more serious damage to the infected fish as they could migrate freely through the visceral organs of the host (Echi et al., 2012). In the present investigation, severe histopathological damage was observed in the liver of infected *T. pectolaris*, which was marked by the presence of migratory track produced by the fluke as well as from host response by chronic inflammation. Infiltration of macrophages and epithelioid cells at the outer rim of the tracks indicated granuloma formation to wall off the damaged area. Similarly, the histopathology of liver damage in *Channa punctata* parasitized with encysted metacercaria of *Euclinostomum heterostomum* exhibited large space of metacercariae cysts and necrosed hepatic tissue (Kaur et al., 2012). The tracks might be generated in hepatic parenchyma by two major mechanisms: the first is the mechanical damage by the prehensile action of the oral sucker together with the mechanical abrasion by spines on the fluke's tegument after they burrow and migrate in liver. Additionally damage to hepatic parenchyma might be due to toxic effects of the excretory-secretory (ES) products of the parasite. The ES products released by metacercaria of Clinostomids were characterized as cysteine protease, which is able to degrade host proteins (Rizvi et al., 2010; Shareef and Abidi, 2014). These products are essential enzyme for the migration and development of the immature flukes. They utilize these substances for host tissue invasion and for their nutrient uptake (Kasny et al., 2009). Eosinophils proliferation frequently accompanies infection with metacercariae of different digenea (Adeyemo and Agbede, 2008; Vankara and Vijayalakshmi, 2013). Adeyemo and Agbede (2008) reported the proliferation of eosinophilic granular cells at the gills of *Oreochromis niloticus* parasitized with metacercariae of *C. tilapia*. Increase in number of eosinophilic granular cells in the area between the host tissue and parasite were recorded in the heart of *Mastacenbelus armatus* infected with Tetracotyle metacercariae (Vankara and Vijayalakshmi, 2013). Milbourne and Howell (1993) suggested that the ES product from fluke functions like interleukin-5 (IL-5), a cytokine stimulating myeloid precursor cells that brings about differentiation and activation of eosinophils. The presence of eosinophilic granular cells at the periphery of the track and on the parasites' tegument observed in this study indicates the host immune response to the metacercariae' ES antigen. As the result of the mechanical damage of toxicity from ES product, the infection with metacercariae of *C. piscidium* could impair the function of the liver and pancreas of the host. The liver is considered to be the important digestive gland which controls several vital functions in fish. It plays a significant role in the metabolism of carbohydrates, proteins, and lipids and storage of glycogen. It is also the site where detoxification takes place (Akiyoshi and Inoue, 2004). Therefore, the massive necrosis of hepatic and pancreatic tissues may disturb the metabolic processes, which in turn, interrupt the entire metabolism of the host, resulting in stunted growth.

In conclusion, the debilitating effects of *C. piscidium* on *T. pectolaris* probably resulted from the pathological changes in liver which can cause growth retardation and significant loss in agriculture production. With the chain of life cycle of the fluke, which needs fresh water snail as the intermediate host and the fish eaten bird as the final host, control of fish infection is difficult since it involves controls of bird and fresh water snail population which may disrupt the balances of the nature and natural resources. Research on prevention, *e.g,* vaccine development and chemotherapeutic needs special attention since Pla-salid is a high valued economic fish of Thailand.

Acknowledgements

This study was supported by Thailand Research Fund (TRF) and Commission on Higher Education; the New Researchers Grant MRG5080181.

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บทคัดย่อ

ความชุกและจุลพยาธิวิทยาในปลาสลิดที่ติดเมตาเซอร์คาเรีย *Clinostomum piscidium* ในพื้นที่ภาคกลางของประเทศไทย

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จุดประสงค์ของการศึกษาครั้งนี้เพื่อจำแนกชนิดของพยาธิที่พบในช่องท้องปลาสลิดและสำรวจความชุกของการติดพยาธิ ความหนาแน่นของพยาธิและพยาธิสภาพในปลาสลิดที่ติดพยาธิดังกล่าว โดยศึกษาในปลาสลิดอายุระหว่าง 8-10 เดือนจากฟาร์มปลาจำนวน 2 แห่ง ที่ตั้งอยู่ในอำเภอบ้านแพ้ว จังหวัดสมุทรสาคร ทำการเก็บปลาสลิดจำนวน 135 ตัว (เพศเมียจำนวน 60 ตัวและเพศผู้จำนวน 75 ตัว) จากฟาร์ม 1 และ 138 ตัว (เพศเมียจำนวน 63 ตัวและเพศผู้จำนวน 75 ตัว) จากฟาร์ม 2 ทำการชั่งน้ำหนักและวัดความยาวในปลาทุกตัวและเปิดผ่าซากเพื่อหาพยาธิ การศึกษาพบพยาธิใบไม้ *Clinostomum piscidium* ระยะเมตาเซอร์คาเรียภายในช่องท้อง โดยพยาธิที่พบไม่มีเปลือกหุ้มและเคลื่อนไหวอย่างเป็นอิสระหรือเกาะที่เนื้อเยื่อไขมันและชั้นนอกของอวัยวะภายในช่องท้อง โดยความชุกของการติดพยาธิและความหนาแน่นของเมตาเซอร์คาเรีย *C. piscidium* ในปลาสลิดเพศเมียจะมากกว่าในปลาเพศผู้อย่างมีนัยสำคัญทางสถิติ ปลาที่ติดพยาธิมีรูปร่างแคระแกรนและน้ำหนักตัวน้อยกว่าปลาที่ไม่ติดพยาธิโดยมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ การสังเกตพยาธิสภาพด้วยตาเปล่าพบจุดเลือดออกสลับกับหย่อมเนื้อตายเป็นทางสีขาวที่พื้นผิวของตับ การสังเกตพยาธิสภาพโดยกล้องจุลทรรศน์พบช่องขนาดใหญ่ซึ่งเป็นทางที่พยาธิเคลื่อนผ่าน ภายในช่องพบเศษเนื้อตายของเนื้อเยื่อตับและตับอ่อนและเม็ดเลือด ล้อมรอบด้วยชั้นของมาโครฟาจและอีพิทีลิออยด์เซลล์ ชั้นนอกพบเม็ดเลือดขาวชนิดลิมโฟไซต์ อีโอซิโนฟิลและไฟโบรบลาสต์ และยังพบอีโอซิโนฟิลอยู่ชิดกับชั้นผิวของพยาธิด้วย การติดพยาธิระยะเมตาเซอร์-คาเรียนี้ทำให้มีพยาธิสภาพที่เนื้อเยื่อตับของปลา กระบวนการเมตาบอลิซึมสารอาหารของตับสูญเสียไป ส่งผลให้ปลาที่ขาดสารอาหารมีรูปร่างแคระแกรนและน้ำหนักตัวน้อย

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