

HUMAN INFECTION OF *CENTROCESTUS CANINUS* IN THAILAND

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Abstract. Examination of fecal samples, collected after treatment with praziquantel, of the persons positive with opisthorchioid eggs residing in the northern provinces of Thailand, was carried out. Five adults *Centrocestus* were recovered in one sample from Doi Saket, Chiang Mai and one adult was found in another fecal sample from Wiang Pa Pao, Chiang Rai. The worms had 26-30 spines arranging in two rows around the oral sucker which agreed with the character of *C. caninus*. This is the first record of natural human infection with *Centrocestus* in Thailand.

INTRODUCTION

More than 20 species of minute intestinal flukes of the family Heterophyidae have been reported in humans (Waikagul, 1985). In Thailand, 4 species of heterophyid flukes were recovered from humans namely, *Haplorchis pumilio*, *H. taichui*, *H. yokogawai* and *Stellantchasmus falcatus* (Radomyos *et al.*, 1994), no report on human infection with *Centrocestus* has been presented.

Similar to the situation of the liver fluke, *Opisthorchis viverrini*, fish is the second intermediate host of the trematode in the family Heterophyidae. *Centrocestus* metacercariae have been reported in several kinds of freshwater fish, *Hampala dispar*, *Puntius gonionotus*, *P. leiocanthus*, *Cyclocheilichthys armatus*, *Mystacoleucus atridorsalis* from Khon Kaen (Srisawangwong *et al.*, 1997), *Abanus testudineus*, *Cyclocheilichthys apogon* from Nakhon Nayok (Waikagul *et al.*, 1990), *C. apogon*, *P. orphoides* from Khon Kaen (Komalamisra and Setasuban, 1989). The life cycle of *Centrocestus* was completely studied in the laboratory by Waikagul *et al.* (1990), starting from cercaria emerging *Melanoides tuberculata* collected from Nakhon Nayok and Saraburi provinces. Metacercariae was recovered from experimental infected fish *Cyprinus carpio* and *Tilapia nilotica* and adult worms were recovered from the posterior half of the small intestine of experimental white rat, 18 days after the infection.

In this study, we report the adult *Centrocestus* found in human fecal samples collected after treat-

ment with praziquantel of two persons from Northern Thailand.

MATERIALS AND METHODS

A survey of liver fluke infection was carried out in the northern provinces of Thailand. Fecal examination by cellophane thick smear method was performed, 40 mg/kg single dose of praziquantel was given to fecal egg positive individuals with opisthorchioid eggs, then 2 hours later, purged with magnesium sulfate. The diarrheal stools were washed and processed individually by sedimentation using the following method: Normal saline solution was added to each stool specimen and stirred until the fecal matter was homogeneously mixed. These were then poured into a 2 liter capacity sedimentation flask and the undissolved matter allowed to settle for 30 minutes, after which the supernate was carefully poured off and discarded. This procedure was repeated 4-6 times until the supernate became clear. The remaining sediments were mixed with an equal volume of 10% formalin then transported to laboratory of the Bangkok School of Tropical Medicine for examination by stereomicroscopy. Collected flukes were stained in Carmine, dehydrated then mounted in Permount for microscopic study.

RESULTS

From a 27 years old man residing in Doi Saket District of Chiang Mai Province, five *Centrocestus*,

one *O. viverrini* and one *Haplorchis taichui* adult flukes were found together with a number of *Enterobius vermicularis* female worms. The second case was a 44 years old woman from Wiang Pa Pao District, Chiang Rai Province, one *Centrocestus* and 70 *H. taichui* were collected from after treatment stools. This paper describes only *Centrocestus* as this is the first record of this worm in human in Thailand.

***Centrocestus caninus* (Leiper, 1913) Yamaguti, 1958**

Body and organs measurements of all specimens are given in micrometers in Table 1.

Description (Fig 1, 2A, B)

Body small, bottle-shape, 432 (372-492) μm long and 120 (96-144) μm wide; covered with scales like spines which decreased in size posteriorly. Mouth terminal; encircled with two alternating rows of spines of 26-30 circumoral spines. Oral sucker elongated oval 44 (37.5 - 50) μm long and 34 (30-37.5) μm wide. Prepharynx absent. Pharynx well developed 31 (25-38) μm long and 24 (20-28) μm wide. Esophagus short. Ceca large, bifurcated about midway between oral and ventral suckers, terminated slightly in front of ovary. Ventral sucker smaller than oral sucker, ratio of oral sucker: ventral sucker 1.2 : 1-1.8: 1; located in the

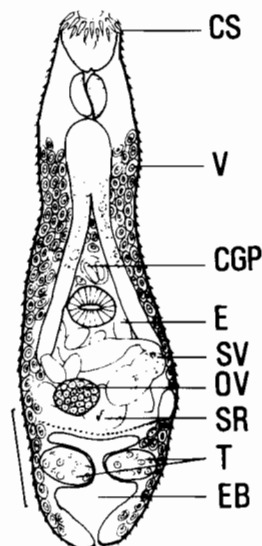


Fig 1—Ventral view of whole mount of adult *Centrocestus caninus* recovered after treatment in stools of humans from Chiang Mai, Thailand. (scale bar = 90 μm)

CS = circumoral spine, CGP = common genital pore, E = egg, EB = excretory bladder, OV = ovary, SR = seminal receptacle, SV = seminal vesicle, T = testes, V = vitellaria

middle of the body. Testes oval, opposite in posterior body, half encircled by x arms of excretory bladder; left testis 30 (22.5 - 37.5) μm long and 33

Table 1

Measurements (μm) of *Centrocestus caninus* recovered from human fecal specimens collected after treatment with praziquantel.

Organs	length/width						Average
	1	2	3	4	5	6	
Body	468/120	408/120	384/144	492/144	468/96	372/120	432/120
Oral sucker	40/37.5	42.5/37.5	40/37.5	50/37.5	37.5/37.5	37.5/30	43.75/33.75
Pharynx	25/22.5	30/20	30/27.5	37.5/27.5	32.5/27.5	32.5/22.5	31.25/23.75
Ventral sucker	-	25/30	22.5/30	30/37.5	27.5/30	32.5/25	27.5/31.25
Ovary	27.5/27.5	30/25	20/27.5	40/37.5	37.5/27.5	35/30	30/27.5
Left testis	22.5/35	25/32.5	22.5/37.5	27.5/35	37.5/35	25/27.5	30/32.5
Right testis	26.25/37.5	20/25	25/27.5	35/52.5	27.5/42.5	27.5/37.5	27.5/38.75
Seminal receptacle	27.5/32.5	20/22.5	-	37.5/45	25/35	20/25	28.75/33.75
Seminal vesicle 1	32.5/55	30/57.5	25/75	27.5/55	-	42.5/25	33.75/50
Seminal vesicle 2	-	37.5/22.5	25/50	-	-	62.5/25	43.75/36.25

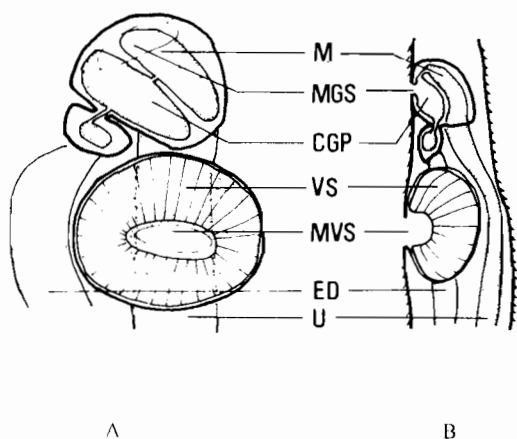


Fig 2—Diagrammatic drawing of genital opening of *Centrocestus caninus*.

A. Ventral view, B. lateral view

CGP = common genital pore, ED = ejaculatory duct, M = metratrem, MGS = mouth of genital sac, MVS = mouth of ventral sucker, U = uterus, VS = ventral sucker

(28-38) μm wide and right testis 28 (20-35) μm long and 34 (25-53) μm wide. Seminal vesicle large, bipartite; first chamber opposite ovary, 34 (25-42) μm long and 50 (25-75) μm wide; second chamber lies transversely between ovary and ventral sucker, 44 (25-62) μm long and 36 (22-50) μm wide; ejaculatory duct lateral to right border of ventral sucker, overlapping and dorsal to right cecum, opens posteriorly into common genital pore by a short and narrow duct (Fig 2A and B). Common genital pore anterior to ventral sucker. Ovary oval, located on the right side of the middle of posterior half of the body; 30 (20-40) μm long and 28 (25-30) μm wide. Seminal receptacle large, 29 (28-33) μm long and 34 (23-45) μm wide, lies in the middle of the posterior half of the body dorsal to ovary, between seminal vesicle and anterior border of excretory bladder. Uterus short, coil between ovary and seminal vesicle, filled with 5-29 eggs; metratrem arises dorsal to ventral sucker, and join common genital pore by short narrow duct (Fig 2A and B). Vitelline follicles large, scattered laterally from posterior end to posterior border of pharynx and dorsal to bifurcation region; vitelline ducts run transversely from each side to form common vitelline duct anterior to anterior border of excretory bladder. Egg oval, 33 (30-35) μm long and 14 (13-16) μm wide, shell surface with lattice design,

yellowish brown, operculum distinct. Excretory bladder x-shaped with short posterior tube; excretory pore terminal.

Host : man
 Location : in feces after treatment
 Locality : Chiang Mai and Chiang Rai Provinces, North Thailand.
 Deposition : Mahidol University, Faculty of Tropical Medicine, Department of Helminthology, Bangkok. MUTMHM 40001
 Meguro Parasitological Museum, Tokyo. MPM 19716

DISCUSSION

The genus *Centrocestus* was erected by Looss (1899) and deposited *Distomum cuspidatum* Looss, 1896 as type species, *C. cuspidatus*. The second species of the genus was described as *C. cuspidatus* var. *caninus* by Leiper (1913) which later had been regarded as synonym of *C. cuspidatus* by Ransom (1921), however Yamaguti (1958) raised it to a valid species, *C. caninus*. Onji and Nishio (1916) reported a new species of worms for experimental cat, *Stephanopirum longus*, which later on was placed as synonym of *C. caninus* by Yamaguti (1958). Tanabe (1922) had described a new species, *Stamnosoma armatum* from experiment in man, cat, dog, rat, mouse and rabbit. Price (1932) recognized *Stamnosoma* as synonym of *Centrocestus*, and *C. armatus* is a valid species. *Stamnosoma nycticoracis* was described from birds, *Nycticorax nycticorax*, *Ardea cinerea*, *Phalacrocorax carbo*, *Milvus migrans* and cat by Izumi (1935) and this species, *C. nycticoracis* had been placed as synonym of *C. armatus* by Yamaguti (1958). Nishigori (1924) has described a new species *S. formosanus* from experimental dog in Formosa; as Price (1932) regarded *Stamnosoma* to be identical to *Centrocestus*, this species become *C. formosanus*. Faust and Nishigori (1926) suggested that *C. cuspidatus caninus* was the synonym of *S. formosanus*. Chen (1942) and Kobayashi (1942) referred to the name *C. formosanus*, however, Yamaguti (1958) placed *C. formosanus* as synonym of *C. caninus* but later publication still retained the name *C. formosanus* (Ito, 1964; Yanohara *et al*, 1987). Kurokawa (1935) described the worm obtained after treatment from

man in Hiroshima as *C. formosanus* var *kurokawai*, later Yamaguti (1958) raised to valid species *C. kurokawai*. Kobayashi (1942) reported two new species *C. yokogawai* and *C. polyspinosus* from experimental dog infected with metacercariae from naturally infected fish, *Ophicephalus maculatus*, *Anabas testudineus*, *Clarias fuscus* and *Misgurnus* sp. from Hainan Island. Yamaguti (1958) placed *C. yokogawai* as synonym of *C. caninus*, but *C. polyspinosus* remain valid species. Mishima (1959) has reported *C. asadai* recovered from after treatment stool of himself taking metacercariae from various brackish water, *Acanthogobius flavimanus*, *Leiognathus argentea*, *Hyporhamphus sajori* from Japan.

The differentiation of *Centrocestus* was based on the number and size of circumoral spines; the margin form of ovary and testes; the size, number of eggs in uterus and the ridges pattern on egg shell surface, level of ceca ends; shaped of excretory bladder; the presence of prephargnx (Kobayashi, 1942; Chen, 1942; Morozov, 1964). Some of these features are varied according to worm age or the state of preparation of specimens (Chen, 1942). The worms may be grouped according to their number of circumoral spines as : 1). 26-36 spines, *C. yokogawai*, *C. caninus*, *C. longus*, *C. formosanus*, *C. cuspidatus*, *C. asadai*; 2). 38-48 spines, *C. nycticoracis*, *C. kurokawai*, *C. armatus* and 3). 50-60 spines, *C. polyspinosus*. In the first group, *C. cuspidatus* differs from other species in having V shape excretory bladder (Morozov, 1964) and vitellaria arrange in two lateral groups, not appeared on dorsal of anterior body, the other species are similar and according to the priority, the name should be given as *C. caninus*. Similarly in the second group, priority is given to *C. armatus*. In this regard, only four species should be regarded as valid species as follows:

Centrocestus cuspidatus (Looss, 1896) Looss, 1899

C. armatus (Tanabe, 1922) Price, 1932

Synonyms : *Stamnosoma armatum* Tanabe, 1922

S. formosanum kurokawai Kurokawa, 1935

Stamnosa nycticoracis Izumi, 1935

C. caninus (Leiper, 1913) Yamaguti, 1958

Synonyms : *C. cuspidatus caninus* Leiper, 1913

Stephenopirum lengus Onji and Nishio, 1916

Stamnosoma formosanum Nishigori, 1924

C. yokogawai Kobayashi, 1942

C. asadai Mishima, 1959

C. polyspinosus Kobayashi, 1942

The number of circumoral spines of the specimens in this study were 26-30 which agreed with *C. caninus*. This is the first record of natural infection by this species in man in Thailand. This species (as *C. formosanus*) had been reported in human experimentally and naturally from Taiwan. Another species, *C. armatus* has been naturally reported from humans in Korea and Hiroshima, Japan (as *C. f. kurokawai*) (Hong *et al*, 1988; Kurokawa, 1935).

The life cycle of *C. caninus* was completely elucidated and several species of fish have been reported as second intermediate hosts, *ie Cyclocheilichthys* spp, *Puntius* spp, *Hampala dispa*, *Mystacoleucus atridorsalis*, *Anabus testudineus*. (Waikagul *et al*, 1990; Komalamisra and Setasuban, 1989; Srisawangwong *et al*, 1977). Natural infection in rat of *Centrocestus* sp was also reported from Chiang Mai Province (Namue and Wong-sawad, 1997). In this regard, source of infection of this species is available naturally and readily for human infection. The metacercaria of *Centrocestus* is encysted mostly in gills of fish; in heavily infected fish, metacercariae may be present under the scale of muscle but in smaller number than in the gills. As gills and scales are discarded in normal practice of fish cooking, number of infected persons are scarce. Moreover, number of eggs in uterus of this worm is small (5-29 eggs), the chance of seeing worm eggs in fecal examination is low in single infection individual. In addition, frog has also been reported as second intermediate host harboring metacercaria of *Centrocestus* by Chen (1942). Naturally, transmission of the worm can be both through infected fish and frog.

REFERENCES

- Chen HT. The metacercaria and adult of *Centrocestus formosanus* (Nishigori, 1924) with notes on the natural infection of rats and cats with *C. armatus* (Tanabe, 1922). *J Parasitol* 1942; 28 : 285-98.
- Faust EC, Nishigori M. The life cycles of two new species of Heterophyidae parasitic in mammals and birds. *J Parasitol* 1926; 13 : 91-128.
- Hong SJ, Seo BS, Lee SH, Chai JY. A human case of *Centrocestus armatus* infection. *Korean J Parasitol* 1988; 26 : 55-60.

- Ito J. *Metagonimus* and other human heterophyid trematodes. In: Morishita K, Komiya Y, Matsubayashi H, eds. Progress of Medical Parasitology in Japan, Vol 1. Tokyo: Meguro Parasitological Museum, 1964: p 318-93.
- Izumi M. A new species of genus *Stamnosoma* and its life history. *Tokyo Iji Shinshi* 1935; 59 : 2423-30 (in Japanese).
- Kobayashi H. Studies on trematoda in Hainan Island. II Trematoda found in the intestinal tracts of dogs by experimental feeding with certain fresh and brackish water fish. *Jpn J Med Sc* 1942; 6 : 187-227.
- Komalamisra C, Setasuban P. Heterophyid flukes and *Opisthorchis viverrini*: intensity and rate of infection in cyprinoid fish from an endemic focus in northeast Thailand. *J Trop Med Parasitol* 1989; 12: 22-8.
- Kurokawa T. On a new species of *Stamnosoma* found from the man. *Tokyo Iji Shinshi* 1935; 59 : 293-8 (in Japanese)
- Mishima T. Studies on a new species of the genus *Centrocestus* and its life cycle. *Tokyo Iji Shinshi* 1959; 76 : 291-9.
- Morozov FN. Key to the species of the genus *Centrocestus* Looss, 1899. In: Skrjabin KI, ed. Keys to the trematodes of animal and man. Urbana: University of Illinois Press. 1964; p 54.
- Namue C, Wongsawad C. A survey of helminth infection in rats (*Rattus* spp) from Chiang Mai Moat. *Southeast Asian J Trop Med Public Health* 1997; (suppl 1): 179-83.
- Nishigori M. On a new trematoda, *Stamnosoma formosanum* n sp, and its history. *Taiwan Igakkai Zasshi* 1924; 234 : 181-228 (in Japanese).
- Price EW. On the genera *Centrocestus* Looss and *Stamnosoma* Tanabe. *J Parasitol* 1932; 18 : 309.
- Radomyos P, Radomyos B, Tungtrongchitr A. Multi-infection with helminths in adults from northeast Thailand as determined by post-treatment fecal examination of adult worms. *Trop Med Parasitol* 1994; 45 : 133-5.
- Srisawangwong T, Sithithaworn P, Tesana S. Metacercariae isolated from cyprinoid fishes in Khon Kaen District by digestion technic. *Southeast Asian J Trop Med Public Health* 1997; (suppl 1) : 224-6.
- Tanabe H. Studies on the trematoda of fresh water fishes as the second host (1). On a new intestinal fluke, *Stamnosoma armatus* ng, n sp. *Kyoto Igakkai Zasshi* 1922; 19 : 5-18 (in Japanese).
- Waikagul J, Visiassuk K, Sanguankait S. Study on the life-cycle of *Centrocestus caninus* (Leiper, 1913) (Digenea : Heterophyidae) in Thailand. *J Trop Med Parasitol* 1990; 13 : 50-6.
- Waikagul J. A review of heterophyid flukes. *Chula Med J* 1985; 29, 1131-8.
- Yamaguti S. Systema helminthum. Digenetic trematodes Part 1. Vol 1. New York: Interscience Publishers 1958; p 706-7, 872-3.
- Yanohara Y, Nojima H, Sato A. Incidence of *Centrocestus fromosanus* infection in snails. *J Parasitol* 1987; 73 : 434-6.