

Infection of *Clinostomum complanatum* (Rudolphi, 1814) (Trematoda : Digenea) metacercaria in goldfish (*Carassius auratus*) cultured in Korea

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Many encysted metacercariae of *Clinostomum complanatum* (Rudolphi, 1814) were found in the flesh near the skin of goldfish (*Carassius auratus*) cultured at a fish farm in Chonbuk Province, Korea, in the summer of 1995. The mortality reached about 7% from July 10 to August 20. Encysted and excysted metacercariae were $700\sim 877\times 700\sim 775\ \mu\text{m}$ and $2.4\sim 5.5\times 0.7\sim 1.37\ \mu\text{m}$ in size, respectively. The parasites looked yellowish due to the yellow particulate matter in the intestine. Of 276 fish examined, 234 (84.8%) were infected with a mean intensity of 216.6 (range 1~1,214) parasites. Fish of 2.0~2.5 cm in body length were most frequently (94.3%) infected but those of 3.1~3.5 cm were the least infected (75.27%). The isthmus was most heavily infected. There was no difference in prevalence between two forms of goldfish, Gyariko and Ryugum. The sites of infection were swollen and recognized as yellow spots of 0.4~0.8 mm in diameter. Fish infected with more than 200 metacercariae became moribund with listless swimming and finally died.

Key words : Trematoda, *Clinostomum complanatum*, *Carassius auratus*, Pathogenicity

Clinostomum complanatum (Rudolphi, 1814) (Trematoda : Clinostomidae) often causes growth retardation and host mortality by infecting the muscle of freshwater fishes (Hoshina *et al.*, 1965 ; Lo *et al.*, 1981, 1985). This species uses fish-eating birds as definitive hosts (Ukoli, 1966 ; Yamaguti, 1871, 1875) and its metacercariae, known as "Yellow grubs", occur in many species of freshwater fishes. Aquatic snails are known to be its first intermediate hosts.

Interestingly, this parasite has been reported to occur in the pharynx of the humans (e.g., Hirai *et al.*, 1987 ; Yoshimura *et al.*, 1991 ; Isobe *et al.*, 1994 ; Chung *et al.*, 1995).

In the Far East Asia, *C. complanatum* is known to occur in freshwater fishes of Japan and Taiwan. In Japan, it has been found as metacercariae in several species of crucian craps (*Carassius* spp.), pike gudgeon (*Pseudogobio esocinus*), topmouth gudgeon (*Pseudorasbora parva*), common bittering

(*Rhodeus lanceolatus*, as *Acheilognathus intermedia*), rose bittering (*Rhodeus ocellatus*), common carp (*Cyprinus carpio*), Japanese dace (*Tribolodon hakonensis*, as *Leuciscus hakonensis*), Asian pond loach (*Misgurnus anguillicadatus*), and Japanese perch (*Lateolabrax japonicus*) (Yamaguti, 1933, 1938; Hoshina *et al.*, 1965; Kagei *et al.*, 1984; Nagasawa, 1991; Aohagi *et al.*, 1992a, 1993b, 1995; Aohagi and Shibahara, 1994).

In Korea, Chung *et al.* (1995a) reported the first human case of *C. complanatum*. The metacercariae of *C. complanatum* was found from the 12 species of freshwater fish collected from natural environment (Chung *et al.*, 1995b), but there are no reporters about this parasite occurring from cultured fish in Korea.

Materials and Methods

The surveyed fish farm was located in the west coast region of Chonbuk Province, Korea. The farm used still-water ponds (30 m long, 20 m wide, and 30~60 cm deep) which transformed from a rice field. Three-month-old goldfish (*Carassius auratus*), 2.0~5.0 cm in body length [BL], were sampled and transported alive to the laboratory on August 21, 1995. The fish were measured for BL and dissected into 6 pieces (Fig. 1) with a surgical knife. These pieces were put in a Petri dish containing 0.7% physiological saline and examined for encysted metacercariae under a dissection microscope. The parasites were removed and mounted between a slide glass and a cover glass, followed by fixation with 10% neutral formalin. These specimens were stained with hematoxylin or acetocarmine and examined for details.

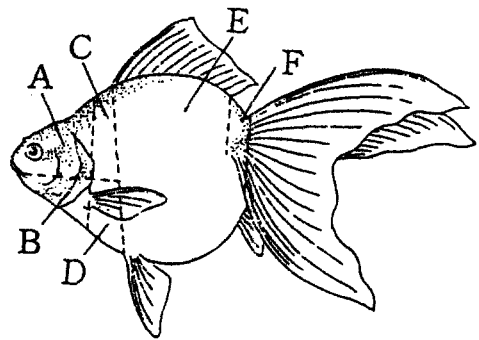


Fig. 1. Goldfish showing the six dissected parts examined for the presence of *Clinostomum complanatum*. A : head ; B : isthmus ; C : thorax ; D : Belly ; E : mid-body ; F : caudal peduncle.

A total of 532 fish, 2.0~4.5 cm BL, were held in tanks at 27°C for 30 days in order to examine the relationship between the parasite number and the host mortality. The behavior of these fish were also recorded.

For elucidating the life cycle at the fish farm, snail were collected from the bottom of the pond, and birds frequently visiting the farm were also observed.

The used terms, prevalence (% occurrence of infected fish in a sample) and intensity (number of the parasite in a host) of infection, follow those defined by Margolis *et al.* (1982).

Results and Discussion

1. Occurrence of the disease

The disease occurred in the lowermost-located pond among several ponds containing about 50,000 goldfish each. Disease fish were first discovered on July 10 and the mortality was about 7% (3,500 fishes) by August 20, 1995. The parasite occurred mostly in Gyariko, one form of goldfish, which

hatched on May 5, 1995. Until the outbreak of the disease, evaporated water was supplemented with ground water every 2~3 days without whole exchange of pond water after the hatching of goldfish, and the growth of phytoplankton was noticeable based on the greenish color of the water. Water temperature ranged from 28~32°C when the disease was found. In Japan, Hoshina *et al.* (1965) reported a similar case of the mortality (50,000 fish) of goldfish and crucian carp caused by *C. complanatum* at a fish farm.

Snails (*Cipangopeludina chinensis malleata*), 1.5~3.5 cm high and 1.0~2.4 cm in diameter, occurred on the bottom of the pond with a density of about 8 snails per m². Numerous another snails (*Lymnaea auricularia coreana*), 1.2~2.3 cm high and 0.7~1.2 cm in diameter, were also found on the grass in the water protruding from the pond and on the grass along the walls of the pond. Although the snails was not examined whether they contained the metacercariae of this parasites, some freshwater snails have been reported to serve as the first intermediate host by several workers. In Taiwan, freshwater snails (*Radix auricularia swinhoei*) serve as the first intermediate host for *C. complanatum* (Lo *et al.*, 1981, 1982). Aohagi *et al.* (1993c) reported that two species of snails (*Lymnaea japonica* and *L. ollula*) are experimentally infected with this parasite. Therefore it is suggested that the snails in our experiment might serve as the first intermediate of this parasite.

Goldfish were observed to be preyed on by common egrets (*Egretta alba*), little herons (*Egretta gazetta*), common kingfishers (*Alcedo atthis*), gray herons (*Ardea cinerea*), and night herons (*Nycticorax nycticorax*). We are certain that these birds

act as the definitive hosts of this parasite because several species of fish-eating birds (genera *Nycticorax*, *Ardea*, and *Egretta*) have been reported to serve as the definitive hosts for *C. complanatum* (Yamaguti, 1933; Lo *et al.*, 1981, 1982; Kagei *et al.*, 1988; Aohagi *et al.*, 1992b, 1993a)

2. Metacercariae

a. Encysted metacercariae (Fig. 2)

The encysted metacercariae were spherical or ellipsoid in shape and easily excysted due to their weak cysts. The encysted metacercariae were 675~850×650~700 μm for unfixed specimens and 700~875×700~775 μm for fixed specimens. According to Aohagi *et al.* (1992a), the cysted metacercariae (called "cysts") removed from Japanese freshwater fishes were larger, being 0.65~2.90 (mean 2.02)×0.65~2.30(1.54) mm. Aohagi *et al.* (1993b, 1995) and Aohagi and Shibahara (1994) obtained similar results.

Fig. 2. Encysted metacercaria of *Clinostomum complanatum*. Scale bar : 100 μm.

b. Excysted metacercariae(Fig. 3)

Excysted metacercariae with round ends were 2.4~3.5 mm long and 0.7~1.37 mm wide, which were slightly smaller than the specimens reported by Yamaguti(1938; 2.95~4.1 mm long, 1.1~1.3 mm wide from Asial pond loach), Aohagi *et al.* (1993b, 1995 : 3.10~6.40[mean 4.63]×0.97~1.96 [1.51] mm from crucian carps, and 2.65~6.28×0.81~1.54 mm from dace and seabass), and Aohagi and Shibahara(1994 : 3.58~6.99×1.09~2.16 mm from crucian carps). Excysted metacercariae actively stretched in physiological saline and looked yellowish due to the yellow particulate matter in the intestine. When the parasites were fixed during their stretching movements, their morphology often changed to a shape with enlarged tail.

100×125 μm. The intestine was quite large with branches and ended as the caecal canal at the posterior end of the body. The ventral sucker was situated at the anterior fourth of the body and measured 500~750×525~750 μm. The primordia of gonads and ducts were located in the middle of the body. Reproductive organs(testes or ovaries) were not well developed. These morphological features and measurements corresponded to those given by Yamaguti(1933, 1938), Lo *et al.* (1982) Aohagi *et al.*(1993b, 1995), and Aohagi and Shibahara(1994).

3. Infection level and site of infection

Prevalence of *C. complanatum* in goldfish is shown in Table 1. A total of 50,677 encysted metacercariae were removed from 234(84.8%) of 276 goldfish examined (mean intensity : 216.6). The highest and the lowest intensity of infection were 1,214 and 1, respectively, found each from 4.0 cm long fish. Fish of 2.0~2.5 cm BL were most frequently(94.3%) infected. Although fish of 3.1~3.5 cm BL were the lowest prevalence(75.3%), the highest mean intensity was recorded in this size class.

The abundance of *C. complanatum* in various body parts of goldfish is shown in Table 2. The isthmus (part B of Fig. 4~5) was the most heavily infected (43.7%), followed by the caudal peduncle (part F). The thorax (part C) was the least infected(4.4%). Some metacercariae were also observed in the tissue near the eye (Fig. 6). Our results are similar to those by Lo *et al.*(1985) but differ from those by Hoshina *et al.*(1965) who found the metacercariae mostly in the muscle near the pectoral fin of crucian carp, followed by near the isthmus and ventral fin.

Fig. 3. Excysted metacercaria of *Clinostomum complanatum*. Scale bar : 1mm

The oral sucker, 125~225×175~300 μm was located at the anterior end of the body. The pharynx was adjoined to the oral sucker, measuring

Table 1. Prevalence and intensity of infection with *Clinostomum complanatum* in goldfish of different sizes from a fish farm in Chonbuk Province, collected on August 21, 1995

Body length(cm)	Number of fish examined	Number of fish infected	Prevalence (%)	Number of parasites	Mean intensity
2.0~2.5	35	33	94.3	6,544	198.3
2.6~3.0	105	95	90.5	16,002	168.4
3.1~3.5	93	70	75.3	19,413	277.3
3.6~4.0	31	26	83.9	7,050	271.2
4.1~	12	10	83.3	1,668	166.8
Total	276	234	84.8	50,677	216.6

Table 2. Occurrence of *Clinostomum complanatum* in different parts of 234 infected goldfish from a fish farm in Chonbuk Province, collected on August 21, 1995.

Parts*	A	B	C	D	E	F	Total
No. of parasites	8,173	22,121	2,238	5,588	4,108	8,449	50,677
%	16.1	43.7	4.4	11.0	8.1	16.7	100

* The parts of the body are shown in Fig. 1.

When the abundance was compared between two strains of goldfish, Gyariko and Ryugum, from the same pond, there was no significant difference between them (Table 3). In both forms, the isthmus was the most heavily infected.

4. Symptoms and mortality of infected fish

Fig. 4-5. Goldfish infected with *Clinostomum complanatum*. Arrows indicate the sites of infection.

Although no difference in overall color was observed between infected and uninfected fish, the sites infected by *C. complanatum* looked inflamed (0.4~0.8 mm in diameter) primarily due to yellow body color of the parasites (Fig. 4). The sites were easily identified by their bumpy appearance as described by Hoshina *et al.* (1965) and Lo *et al.* (1981).

Fig. 6. *Clinostomum complanatum* in the tissue near the eye of goldfish (H-E stained). e : eye ; m : metacercariae.

The mortality of infected goldfish kept in tanks for 30 days is shown in Table 4. Fish of 2.0~3.0 cm BL showed highest mortality (19.1%) but with an increasing size of goldfish the mortality declined. This may be closely associated with the higher intensity level in smaller-sized goldfish (Table 4).

Table 3. Occurrence of *Clinostomum complanatum* in two forms of goldfish, Gyariko and Ryugum, from a fish farm in Chonbuk Province, collected on August 21, 1995

Strain	No. fish examined	No. fish infected	Prevalence (%)	Body part**						Total
				A	B	C	D	E	F	
Gyariko	36	27	75.0	835 (15.7)*	2,403 (45.1)	97 (1.8)	631 (11.8)	352 (6.6)	1,010 (19.0)	5,328 (100)
Ryugum	40	31	77.5	1,161 (21.0)	2,113 (38.3)	295 (5.3)	532 (9.6)	583 (10.6)	838 (15.2)	5,522 (100)

* : % abundance.

** : The parts of the body are shown in Fig. 1

Table 4. Mortality of goldfish infected with various intensity levels of *Clinostomum complanatum*.

Body length (cm)	No. of Fish examined	Mortality (%)	No. of parasites						Total
			1~100	101~200	201~300	301~400	401~500	501~	
2.0~3.0	173	19.1	—	—	4	6	11	12	33
3.1~4.0	167	9.0	—	—	1	3	4	7	15
4.1~	192	2.6	—	—	—	1	1	3	5
Total	532	9.7	—	—	5	10	16	22	53

Fish infected with less than 200 metacercariae looked normal in their activity. However, those infected with more than 200 metacercariae became moribund and their swimming was extremely lethargically near the surface of the water. These fish did not take food and finally died. Many of heavily infected fish also died after the 30-day experiment. Similar mortality of goldfish has been reported in Japan (Hoshina *et al.*, 1965). Lo *et al.* (1985) found that heavy infection of *C. complanatum* experimentally caused the growth retardation of Asian pond loach.

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금붕어에 기생한 *Clinostomum complanatum* (Rud., 1814)의 피낭유충에 관하여

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1995년 8월 전북소재 금붕어 양어지에서 금붕어의 체표 근육에 기생한 흡충, *Clinostomum complanatum*의 피낭 유충을 다량 검출 하였다. 피낭 유충의 크기는 $700\sim 877\times 700\sim 775\ \mu\text{m}$ 였고, 탈낭 유충은 크기가 $2.4\sim 5.5\times 0.7\sim 1.37\ \text{mm}$ 로서, 창자 내부의 황색 과립상 물질 때문에 충체는 황색으로 보인다. 충체의 앞쪽에는 $125\sim 225\times 175\sim 300\ \mu\text{m}$ 의 구흡반이 있고, 복흡반은 $500\sim 750\times 525\sim 750\ \mu\text{m}$ 크기로 충체의 앞쪽 1/4부위에 위치 한다. 어체 1마리당 최고 1,214개, 최저 1개가 기생 되었고, 총 276마리의 조사어에서 84.78% (234마리)의 기생율을 나타냈으며, 기생율이 가장 높은 크기는 체장 2.0~2.5 cm로 94.29%, 가장 낮은 크기는 3.1~3.5 cm로 75.27%이었다. 어체 부위중 B(헝부)가 가장 많이 기생(43.65%)되었고, C(가슴)부분이 가장 적게 기생(4.4%)되었다. 카리코와 유금의 양종간에는 기생율에 큰 차이가 없었다. 본 충의 기생부위는 직경 0.4~0.8 mm의 황색점으로 보이고, 그 부위는 팽윤되어 울퉁불퉁하다. 200개 이상의 피낭유충이 기생된 금붕어는 수면위에 힘없이 유평하거나 쇠약해져서 폐사되었고, 특히 선별후 폐사 개체가 많았다.

Key words : Trematoda, *Clinostomum complanatum*, *Carassius auratus*, Pathogenicity