The Korean Journal of Parasitology
Vol. 31, No. 4, 347-352, December 1993

Larval *Gnathostoma nipponicum* found in the imported Chinese loaches

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Abstract: Six early third-stage larvae of *Gnathostoma nipponicum* were recovered from the muscle of 376 loaches, *Misgurnus anguillicaudatus*, imported from China. They were 614 × 114 μm in average size, almost colorless except brownish intestine, and encircled by about 229 transverse rows of minute cuticular spines. Their head bulbs provided with 3 rows of hooklets, of which average number were 34.5 on the first, 36.7 on the second and 39.7 on the third. Accordingly, it is revealed that the Chinese loach is a natural second intermediate host of *G. nipponicum* and *G. nipponicum* has been distributed somewhere in China. This parasite may infect human when the imported loaches are consumed raw.
Key words: *Gnathostoma nipponicum*, Early third stage larva, Chinese loach

INTRODUCTION

Gnathostoma nipponicum is a common parasite found in esophageal tumor of weasels in Japan. This parasitic nematode was first found by Yoshida (1931) and he erroneously described it as *G. spinigerum*. Thereafter, the morphological features of adult were detailly described by Yamaguti (1941) and later by Miyazaki and Umetani (1950), and the extensive survey of weasels carried out in almost every Prefectures of Japan (Ando *et al.*, 1988b).

The full life history of *G. nipponicum* has been recently known from the results obtained by field survey and experimental infection of various animals with the larvae (Koga and Ishii, 1981; Ando *et al.*, 1992). Arita (1953) and Mabuchi (1957) demonstrated that the second stage larvae hatched from eggs were infective to 3 species of cyclopoid copepods. But they failed to infect the second intermediate hosts such as fish, amphibians and reptiles with the larvae from copepods. Naturally infected larvae were first recovered from snakes, *Rhabdophis tigrinus* (Koga and Ishii, 1981), followed by loaches, *Misgurunus anguillicaudatus* (Ando *et al.*, 1988) and catfish, *Silurus asotus* (Ando *et al.*, 1992).

There have been no reports on the recovery of larval gnathostomes except that of Kim (1973) in Korea. Kim (1973) reported two larval *Gnathostoma* recovered in the abdominal muscle of a snake head, *Channa argus*, from Kimhae, Kyongnam and he identified them as the third stage larvae of *G. spinigerum*. The larval gnathostomes in loaches imported from China were identified as those of *G. hispidum* in Japan (Akahane *et al.*, 1982; Akahane and Mako, 1984). Now, we report the morphological features of early third-stage larvae of *G. nipponicum* from the imported Chinese loaches in Korea.

• Received Sep. 25 1993, accepted after revision Oct. 19 1993.
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MATERIALS AND METHODS

A total of 376 loaches, *Misgurunus anguillicaudatus*, imported from China was purchased in the Chagalchi Fishery Market, Pusan, Korea in March 1992. The loaches were transferred in our laboratory, and their viscera and muscles were isolated and artifitially digested with pepsin-HCl solution in a 36°C incubator. Digested materials were washed with 0.85% saline and were examined under a stereomicroscope to collect helminth larvae. Collected larval gnathostomes were fixed with 10% formalin under the cover glass pressure, cleared in alcohol-glycerin solution and mounted in glycerin-jelly. Mounted specimens were observed and measured under a light microscope with micrometer.

RESULTS

A total of 6 larval gnathostomes and 235 echinostome metacercariae were recovered from 376 loaches. All of the larval gnathostomes were found in the muscles of loaches (Table 1).

The body of the larva, about 674 μm long and 114 μm wide, was almost colorless except brownish intestine, and was encircled by about 229 transverse rows of minute cuticular spines. A pair of lips were located at anterior end, club-shaped esophagus (about 306 μm long) and brownish intestine were followed and anus was opened at the ventral side of posterior end. Two pair of cervical sacs (about 188 μm long) were clearly observed in the region of esophagus (Figs. 1 & 2). The head bulb provided with 3 rows of hooklets, of which average number were 34.5 on the first, 36.7 on the second and 39.7 on the third, increasing posteriorly (Figs. 3 & 4). The detailed measurements of larvae and the comparisons with previous authors were provided in the Table 2 and Table 3.

Table 1. Infection status of the helminth larvae in loaches* imported from China

Helminth larvae	No. (%) of larvae recovered		
	Total	Muscle	Viscera
Gnathostome (3rd. stage larvae)	6	6 (100)	0
Echinostome (metacercaria)	235	187 (79.6)	48 (20.4)

*376 loaches were examined.

DISCUSSION

The nematode of the genus *Gnathostoma* is a well-known causative agent of the creeping eruption in human. Human gnathostomiasis was caused mainly by *G. spinigerum*, however several cases by *G. hispidum*, *G. doloresi* and *G. nipponicum* have been also confirmed (Morita *et al.*, 1984; Nawa *et al.*, 1988; Ando *et al.*, 1988). As for the human cases by *G. nipponicum*, two were found in Japan, and the loaches captured in the dwelling place of patients were suggested as the probable source of infection (Ando *et al.*, 1988).

Since 1980, there has been a considerable increase of human gnathostomiasis cases in Japan, presumably caused by ingesting raw loaches imported from China (Demitsu and Aizawa, 1985). All of the larval gnathostomes collected from the imported Chinese loaches (Akahane *et al.*, 1982; Akahane and Mako, 1984) and the worms recovered from human cases with past history of eating raw Chinese loaches (Morita *et al.*, 1984; Araki, 1986) were identified as those of *G. hispidum*. The Japanese workers have believed that *G. nipponicum* is distributed only in Japan. Now, we found larval *Gnathostoma* from imported Chinese loaches, which were identified as the early third-stage larvae of *G. nipponicum* when their morphological characteristics and measurements were compared with previous records.

Morphological features of *G. nipponicum* larva are quite different from those of the other *Gnathostoma* species distributed in the region of Far East. The most striking difference between *G. nipponicum* and the other species is the number of transverse rows of hooklets on the head bulb. The head bulb of *G. nipponicum* has 3 transverse rows of hooklets and the other species have 4 rows. Accordingly, it is confirmed that the Chinese loach is a second intermediate host of *G. nipponicum*. *G.*

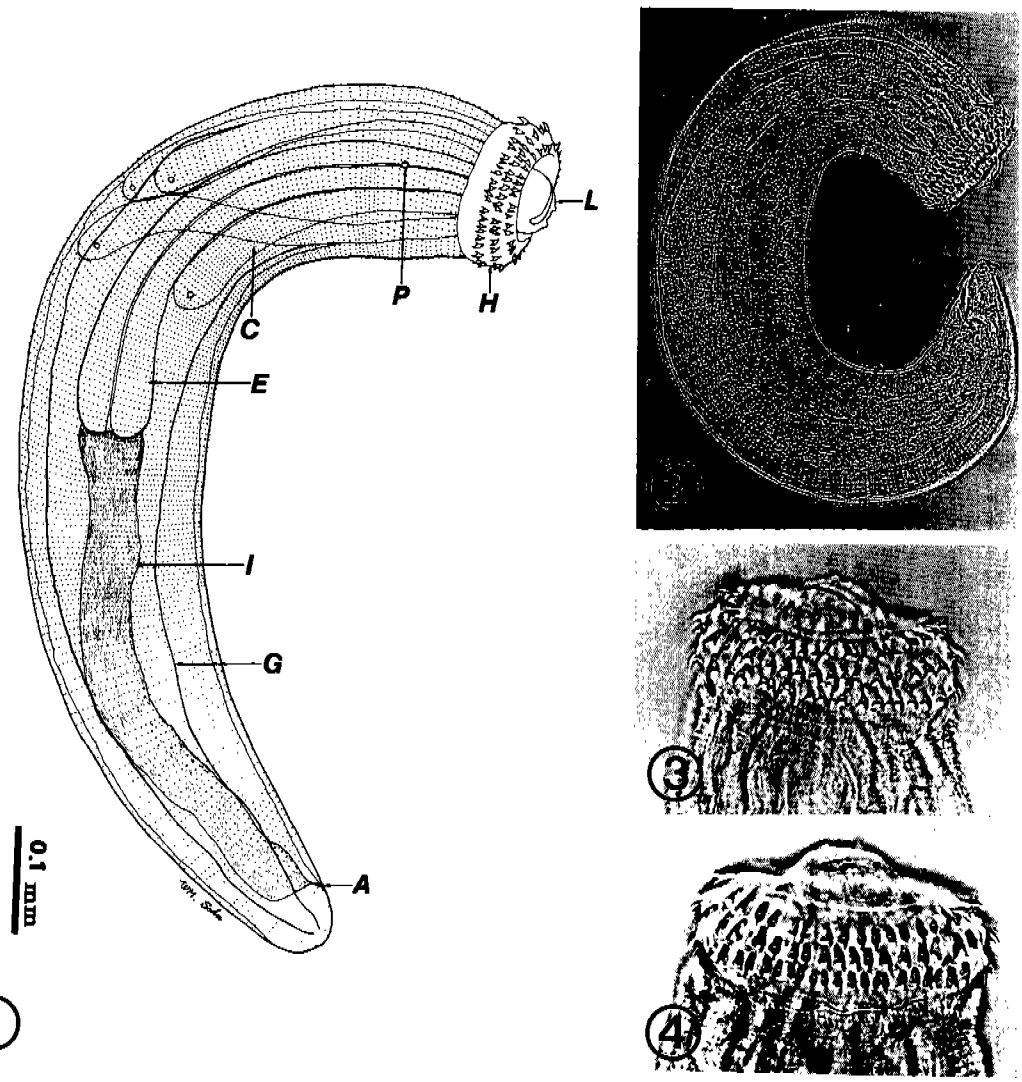

Fig. 1-2. Early third stage larvae of *G. nipponicum* recovered from the imported Chinese loaches (A: anus, C: cervical sac, E: esophagus, G: lateral line, H: head bulb, I: intestine, L: lip, P: cervical papilla). **Fig. 3-4.** Head bulbs of the larval *G. nipponicum*. Note the 3 rows of hooklets, their shape and bases.

nipponicum is also distributed somewhere in China as well as in Japan.

In Korea, two larval *Gnathostoma* were recovered in a *Channa argus* out of 213 examined in Kyongnam and were identified as the third stage larvae of *G. spinigerum* (Kim, 1973), and a male *G. spinigerum* was recovered from a Thai woman with meningoencephalitis and recorded as a imported case (Lee *et al.*, 1988). However no larvae were detected from cyclopoid copepods, tadpoles and loaches (Kim, 1973; Kim, 1983; Koga *et al.*, 1985) and indigenous infection in human or definitive hosts have not been reported yet. However, it is hard to say definitely that this parasite does or does not exist in Korea. Because Korea is located between China and Japan, the presence of *Gnathostoma* is quite plausible.

The present study is the first record on the rare parasite imported through the food-animal. So far, numerous human cases of parasite infection imported from the foreign countries have been reported. However there are few reports on the imported food-animals

Table 2. Measurements[a] of *G. nipponicum* larvae recovered from the loach of China

Larva no.	Body#	Length esophagus	Length cervical sac	Head bulb[b]	No. hooklets on head bulb			Transverse rows of cuticular spines on body
					I	II	III	
1	617 × 102	290	197	31 × 76	35	38	39	217
2	660 × 117	300	185	31 × 86	38	39	42	232
3	610 × 132	343	248	36 × 71	32	35	38	235
4	909 × 140	343	169	31 × 102	38	39	43	248
5	630 × 84	279	153	31 × 76	32	34	38	215
6	620 × 107	281	176	31 × 76	32	35	38	224
Total								
Max.	909 × 140	343	248	36 × 102	38	39	43	248
Min.	610 × 84	270	153	31 × 71	32	34	38	215
Mean	674 × 114	306	188	32 × 81	34.5	36.7	39.7	229

[a] Unit is micrometer. [b] Length × Width.

Table 3. Comparison of the measurements[a] of *G. nipponicum* larvae by authors

Author	Body[b]	Length esophagus	Length cervical sac	Head bullb[b]	No. hooklets on head bulb			Transverse rows of cuticular spines on body
					I	II	III	
Present study (1993)	674 × 114	306	118	32 × 81	34.5	36.7	39.7	229
Ando *et al.* (1988)[c]	829 × 90	347	214	36 × 78	33.4	36.1	40.4	222
Ando *et al.* (1988)[d]	1,161 × 93	388	189	46 × 82	37.0	37.1	41.0	215

[a] Unit is micrometer. [b] Length × Width. [c] 7 worms from naturally infected loaches (3,098 fish)
[d] 10 worms from experimentally infected loaches.

as the transmission vehicle of parasites. The parasites in the imported food-animals are a possible source of human infection, and furthermore they may be a seed of zoonotic prevalence in Korea. In fact, it is questionable that the surveillance on parasites in imported food-animals has been well done. As the imported parasites transmitted by food-animal, *Trichinella spiralis*, *Angiostrongylus cantonensis*, *Toxoplasma gondii* and *Sarcocystis* spp. are of great importance in clinical points of view. Accordingly, we must not consider ourselves fortunate that such imported parasites have been of no public health problem in Korea.

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=국문초록=

중국산 수입 미꾸리에서 검출한 *Gnathostoma nipponicum* 유충의 형태

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1992년 3월에 부산시 중구 남포동 소재 자갈치시장에서 구입한 중국산 미꾸리 376마리에서 6마리의 악구충 유충을 검출하였다. 유충들은 크기가 평균 674 × 114 μm이었고 특징적인 head bulb와 2쌍의 cervical sac(평균 188 μm)을 가지고 있었으며 전 체표면에는 미세한 가시가 질서정연하게(평균 229열) 배열되어 있었다. 충체의 전단에는 구순이 돌출되어 있었고 그 뒤로 식도(평균 306 μm)와 장이 이어졌으며 충체 후단 근처의 복측에 항문이 개구하였다. Head bulb에는 소구(hooklet)가 평균 34.5개, 36.7개 및 39.7개씩 3열로 배열되어 있었다. 충체의 계측치 및 형태학적 특징을 근거로 *Gnathostoma nipponicum*의 제3기 유충으로 동정하였으며 중국산 미꾸리가 이 선충의 제2중간숙주임을 확인하였다. 이러한 수입 식용동물들이 국내에는 없는 새로운 기생충 질환의 감염원이 될 수 있을 것으로 판단된다.

(기생충학잡지 31(4): 347-352, 1993년 12월)