A SURVEY OF *GNATHOSTOMA* LARVAE IN FRESH WATER FISH IN THE VALLEY OF THE YANGTZE RIVER AND MORPHOLOGICAL CHARACTERISTICS OF THE RECOVERED LARVAE

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Abstract. Investigations of the prevalence of larval gnathostomes in fresh water fishes were carried out at the southeastern Yangtze Valley, People's Republic of China, in the periods of October 1989 and November 1990. Fishes were collected from Shanghai, Chenchiang, Nanching, Chiuchiang and Nanchang districts in 1989. Additional sampling in Shanghai district was done at Kunshan, Tien-shanfu, Chingpu and Nanhui. Species of fishes collected were *Channa argus* (110), *Siniperca chuatsi* (24) and *Silurus asotus* (2). Muscle tissue of the fishes was dissected into small pieces, sliced and then examined under a dissecting microscope. The viscera were pooled by species in groups of 4 or 5 individuals, homogenized, and were then digested overnight in artificial gastric-juice at 37°C. Four encysted larvae were recovered from the muscle tissue of four *C. argus*. Thirty-four larvae were obtained from digestion of viscera. A total of 38 larvae were recovered. Eighteen of 38 larvae were examined morphologically and they were able to be divided into three types by their body length; 5 early third-stage larvae (0.58-0.86 mm), 12 third-stage larve (1.12-2.61 mm), and one advanced third-stage larva of 4.86 mm. Light and scanning electron microscopy revealed that the former two types had characteristics of *Gnathostoma hispidum* and the last one had those of *G. spinigerum*. In 1990, we investigated fish near Hongtze-hu and Tai-hu lakes. A total of 553 fishes belonging to 12 genera and 12 species were examined. Seventeen larvae were recovered from the viscera of *G. argus* and *Monopterus albus*. These larvae were identified as *G. hispidum*.

INTRODUCTION

Since the early 1900s, peculiar cutaneous lesions have been sporadically found among Japanese living along the Changchiang River. Most cases have occurred in the southeastern district of this valley in mainland China (Yamada, 1902). This condition was termed Changchiang edema and was believed to develop after eating raw fresh water fish. In 1944, Komaya *et al* surgically removed gnathostome larvae from subcutaneous tumors of two patients in China, and identified the worms as *Gnathostoma spinigerum* Owen, 1836. Subsequently Komaya *et al* (1945) examined several fishes from the same district and also found the etiological agent of this disease. Oturu and Katagiri (1958) examined three species of pisces bought in a market in Shanghai, China. Recently, many gnathostome larvae were found in 14 species of fishes captured around Lake Hongtze-hu near Nanchin, China (Lin and Chen, 1986). According to their report, the advanced third-stage larvae of *G. spinigerum* were most frequently found in the viscera of the fishes. The same developmental stages of this species were recovered from muscle tissue in fishes from other countries including the Philippines (Africa *et al*, 1936), Thailand (Daengsvang *et al*, 1966; Setasuban *et al*, 1991) and Japan (Miyazaki and Umetani, 1951; Kikuchi 1956). Because of differences in parasitic habitat in fishes between China and other parts of Asia, samples were reexamined in this study to determine the species of gnathostome from this part of the world.

MATERIALS AND METHODS

Field investigations were carried out in the period between October and November, 1989 and 1990. In the first year, we collected three species of fishes from five districts along the valley of the Changchiang (Yangtze) River including Nanchan, Chiuchiang, Nanching, Chenchiang and Shanghai. Collection in the Shanghai district also included Kunshan, Tienshanhu, Chingpu and Nanhui. A total of 136 fishes were collected including *Channa*

argus (110), *Siniperca chuatsi* (24) and *Silurus asotus* (2). The animals were killed, and the viscera from each fish were separated from the body. Skin, bones and spines were discarded. Muscle tissue was dissected into small pieces and sliced. These slices were thoroughly examined piece by piece being pressed between two glass plates under a dissecting microscope. Viscera from 4 or 5 individuals of the same species were mixed together, homogenized, and then digested overnight in an artificial gastric juice (pepsin 0.2 g, HCl 0.7 ml in 100 ml water) at 37°C. The sediments were washed several times in tap water and were examined under a dissecting microscope.

In 1990, 553 fishes of 12 species were examined. These were collected around the Hongtze-hu and Tai-hu Lakes. They were located either side of the huge river. Species exermined included *Channa argus* (22), *Cyprinus carpio* (8), *Carassius aurutus* (83) *Monopterus albus* (103), *Erythroculter dabryi* (27), *Hypophthalmichtys molitrix* (4), *Tilapia nilotica* (10). *Abbotina rivularis* (90). *Acanthorhodeus chankaensis* (34), *Misgurnus anguillicaudatus* (14), and *Parabramis pekinensis* (1). Examination of these fishes was done as described previously. Some individuals in these collections were too small to separate muscle tissue from the viscera. These fishes were grouped by species, chopped into small pieces and digested as described earlier. Seven of 18 larvae obtained in 1990 were examined by light and scanning electron microscopies (SEM), Specimen preparations for SEM were done by conventional methods.

Since feline and canine mammals are natural definitive hosts of *G. spinigerum*, we exermined the stools of 43 domestic cats from Hongtze-hu district for gnathostome eggs. In addition 21 cats were anesthetized and necropsied to examine adult worms parasitic in a tumor of the stomach wall.

RESULTS

One hundred and thirty-six fishes were collected along the Yangzte Valley from Nanchang to Shanghai in 1989 for examining gnathosthome larvae. Fifty-eight of these fishes were collected in four minor districts of Shanghai (Table 1). Thirty-eight larvae were recovered in total (Table 2). Thirty-four out of 38 were obtained from the viscera. While only four were recovered from muscle tissue of 4 *Channa argus* from Chiuchiang and Chenchiang areas. All four of these larvae were encapsulated in the muscle (Fig 1). The cyst measured about 0.8 × 0.8 mm. *C. argus* in Chuichiang harbored the highest number of larvae (15 larvae from 16 fish) and that of Shanghai showed the lowest prevalence (only 1 from 56 fish). Eighteen of 38 larvae obtained were used for morphlogical observation. Five larvae were 0.58-0.86 mm long (group A), 12 were 1.12-2.61 mm (group B) and only one was 4.86 mm long. Larvae in group A were the early third-stage, while the largest one was the advanced third-stage (Table 3). Group B larvae were intermediate in development. The remaining 20 viable larvae in group B were experimentally infected to rats in which they grew to the advanced third-stage (unpublished data). All larvae had four transverse rows of hooklets on the anterior extremity of the head-bulb. By SEM, the larvae were found being provided with a pair of cervical papillae, postrior body papillae and a ventrally situated excretory pore. Minute cuticular spines were spaced along the transverse striations. Morphological charactristics are shown in Table 4. Larvae in groups A and B (including the larvae harvested after experimental infection to mammals) were identified as *Gnathostoma hispidum*. The single larva which was tentatively called group C was identified as *G. spinigerum* according to the characteristics of 4 species of gnathostome larvae reported by Koga *et al* (1995).

Table 1

No. of fishes examined in the Yangtze Valley in 1989.

Locality	Fish species	No. of fishes examined
Shanghai	*Channa argus*	56
	Silurus asotus	2
Chenchiang	*C. argus*	13
Nanching	*C. argus*	10
Chiuchiang	*C. argus*	16
	Siniperca chuatsi	10
Nanchang	*C. argus*	15
	S. chuatsi	14
Total		136

Table 2

No. of gnathostome larvae recovered from fishes in China in 1989.

Locality	Fish species	Dwelling spots	No. of larvae obtained (stage)
Shanghai	*Channa argus*	muscle	0
		viscera	1
	Sirurus asotus	muscle	0
		viscera	0
Chenchiang	*C. argus*	muscle	1 (encysted)
		viscera	5 (EL_3, 1;$Ad.L_3$, 1)
Nanching	*C. argus*	muscle	0
		viscera	3 (EL_3, 1)
Chiuchiang	*C. argus*	muscle	3 (encysted)
		viscera	15 (EL_3, 1)
	Siniperca chuatsi	muscle	0
		viscera	6
Nanchang	*C. argus*	muscle	0
		viscera	4 (EL_3, 2)
	S. chuatsi	muscle	0
		viscera	0
Total			38

EL_3: Early third stage larvae
$Ad.L_3$: Advanced third-stage larva

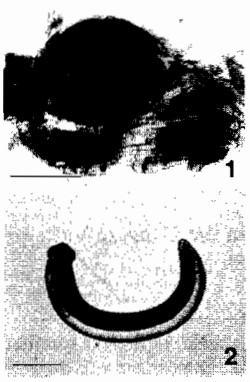

Fig 1– A larva encapusulated in the muscle of fish *Channa argus* captured in Chenchiang. Scale bar = 500 μm.

Fig 2– An early third-stage larva recovered from the viscus of *C. argus* in Nanchang. Scale bar = 100 μm.

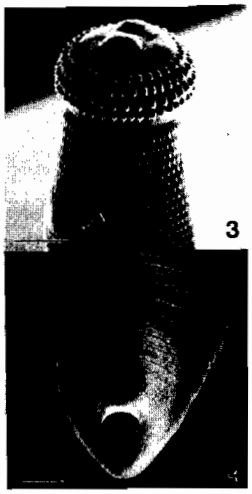

Fig 3– Scanning electron micrograph of anterior region of *Gnathostoma hispidum* larva. Arrow head indicates the cervical papilla. Scale bar = 40 μm.

Fig 4– SEM of tail end of the larva. Cloacal opening is clearly seen on the ventral side. Minute cuticular spines are sparsely evident up to the tail extremity. Scale bar = 15 μm.

In 1990, one of 3 larvae were found in 3 *C. argus* in Tai-hu, was encapsulated in the muscle (0.8 × 0.95 mm) and the other two were recovered from the viscera. In addition, 7 larvae were recovered from the viscera of 78 *M. albus*. In Hongtze-hu, 8 larvae were recovered from the viscera of 26 *M. albus*. Larvae were not found in other fish species from the lakes. Only 18 larvae in total were harvested in the second year. Seven larvae were morphologically examined and they were all found to belong to groups A and B. Representatives of these groups were *Gnathostoma hispidum* by their morphological charactristics. No larva having charactristics of *G. spinigerum* was found in 1990. The remaining 11 larvae in groups A and B were

again infected to rats in which the advanced third-stage of *G. hispidum* were encapsulated in muscle. 43 domestic cats living around Hongtze-hu district showed no adults or eggs of gnathosomes by fecal and autopsy examinations.

DISCUSSION

According to the reports by Komaya *et al* (1945) and Oturu and Katagiri (1958), the advanced third-stage larvae of *G. spinigerum* were frequently found in *C. argus, S. chuatsi* and *Odontotutis obscurus* from the Yangtze valley, China. Furthermore, the larvae were mainly parasitic in the viscera of the fishes. Recently Lin and Chen (1986) examined 28 species of fishes (503 in total) in Hongtze-hu district in Jiangsu Province and found the advanced third-stage larvae of *G. spinigerm* in 14 species. The most commonly infected species were *M. albus, O. obscurus, Pseudobagrus fulvidraco* and

Table 3

Measurements of 18 gnathostome larvae recovered in 1989.

Locality	Fish species	Size of larvae	
		Length	× width(mm)
Chenchiang	*C. argus*	0.86	0.073
		1.79	0.160
		2.61	0.210
		4.86	0.330#
Nanching	*C. argus*	0.79	0.100
Chiuchiang	*C. argus*	0.82	0.080
		1.12	0.110
		1.15	0.135
		1.46	0.190
		1.64	0.160
	S. chuatsi	1.16	0.190
		1.69	0.120
		1.74	0.210
		1.75	0.190
		1.80	0.180
		2.11	0.190
		0.575	0.070
Nanchang	*C. argus*	0.685	0.065

Advanced third-stage larva

Table 4

Morphological characteristics of gnathostome larvae by size grouping.

Types	Body length	No. of hooks on head-bulb				Locations of		No. of transverse striations
		I	II	III	IV	Cervical papillae	Excretory pores	
A	575-860	39	39	40	42	9-12th#	17-20th	201
B	1,120-2,610	39	37	41	43	9-12th	18-20th	191
C	4,860	38	46	46	50	13-14th	25-26th	253

The ordinal numbers show locations of the transverse striation

C. argus. These species were heavily infected with larvae (1,557 in total), but organs and tissues where they were found were not reported. In the present study, comparatively fewer larvae were obtained. This was especially true in Shanghai, where only one larva was recovered from 56 *C. argus*. This indicates a sharp drop in prevalence of this gnathostome from 53% to 2% since the study by Komaya *et al*, (1945).

Larvae we collected this time were as nearly small as the early third-stage larvae, very few of which were living in muscle, while those described in other reports were predominantly the advanced third-stage larvae of *G. spinigerum* (mostly living in viscera). We identified almost all of our larvae (A and B) as *G. hispidum* according to charactristics described by Koga *et al* (1987, 1988, 1994). These results suggest that *G. hispidum* currently prevails at only moderate levels in the Yangze Valley.

Recently, human gnathostomiasis has sporadically occurred in Japan (Araki and Morita, 1981; Morita *et al* 1984; Ando, 1992). The patients had eaten raw fresh water fish, *M. anguillicaudatus*, imported mainly from China. This fish can be purchased easily in city markets in Japan and frequently have many small, early third-stage larvae in their viscera (Akahane *et al*, 1982; Kondo *et al*, 1984; Koga *et al*, 1985). This small parasite was later identified as *G. hispidum*. This observations support our findings in mainland China.

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