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ANISAKIDAE LARVAE FOUND IN MARINE FISHES AND SQUIDS FROM THE GULF OF TONGKING, THE EAST CHINA SEA AND THE YELLOW SEA

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SUMMARY: Marine fishes and squids caught in the Gulf of Tongking, the East China Sea and the Yellow Sea were dissected, and many Anisakidae larvae were found in them. The larvae of *Anisakis simplex* (*Anisakis* Type I larvae), the most important causative agent of anisakiasis, was found in about 30% of fishes and squids caught in the Gulf of Tongking and in about 60% of those caught in the East China Sea and the Yellow Sea. Larvae belonging to three other genera than *Anisakis* — *Raphidascaris* or *Raphidascaroides*, *Hysterothylacium*, and *Terranova* (Cannon Type I) — were also detected.

INTRODUCTION

Since Van Thiel (1) reported the discovery of ascaridoid nematode larvae of genus *Anisakis* (Anisakidae, Nematoda) in a patient suffering from acute abdominal pain and the worms were found to be a causative agent of this

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syndrome, many biological studies on Anisakidae have been conducted throughout the world, especially in Japan (2-9).

In China, however, little has been studied on the parasite, with only exceptions of the work by Hsu et al. (10) and by one of the present authors (11,12). One of the latter two reports also dealt with the investigation on Anisakidae larvae found in marine fishes and squids, but it was preliminary and incomplete (11). Therefore, the present authors intended to advance their work to clarify the state of *Anisakis* infection among fishes captured off the Chinese continent and to gather basic knowledge on human anisakiasis.

MATERIALS AND METHODS

Marine fishes and squids captured in the East China Sea and the Yellow Sea were provided by Shanghai Marine Fishery Company between March and July, 1985, and those captured in the Gulf of Tongking by Guangzhou Marine Fishery Company during December, 1986.

The fishes and squids were measured for the body weight and length and they were dissected to detect Anisakidae larvae. The collected larvae were fixed with a heated 70% ethanol solution and cleared as usual with a 70% ethanol/glycerine mixture (9:1). The morphological features of the worms were observed under a microscope, and the size of each organ of the worms was measured. The worms were identified from their morphological characteristics.

RESULTS

Anisakidae Larvae Found in Marine Fishes and Squids Captured in the Gulf of Tongking

Thirty-one species of marine fishes and squids were investigated for the presence of Anisakidae larvae. The detected *Anisakis simplex* larvae (*Anisakis* Type I larvae) were measured and morphologically examined (Table I). The larvae were identified from the morphological data obtained in reference to the standard published by Koyama et al (2).

In 15 species of fishes tested, the infection with *Anisakis simplex* larvae was found (Table II). The number of fishes and squids examined was 140, and the number of those infected with the larvae 41. The infection rate was 29.3%.

Table I. Measurements* of *Anisakis simplex* larvae from fishes of the Gulf of Tongking

Host	Number of the larvae examined	Length	Width	Oesophagus		Tail
				Muscular	Glandular	
<i>Psettodes erumei</i>	20	18.33 (14.24-22.00)	0.34 (0.30-0.46)	1.61 (1.33-1.78)	0.68 (0.56-0.83)	2.29 (2.03-2.54)
<i>Trichiurus haumela</i>	20	19.12 (15.33-25.57)	0.34 (0.30-0.39)	1.58 (1.31-1.84)	0.71 (0.61-0.79)	2.29 (1.95-2.74)
Total	40	18.73 (14.24-25.57)	0.34 (0.30-0.46)	1.60 (1.31-1.84)	0.70 (0.56-0.83)	2.29 (1.95-2.74)
						0.09 (0.06-0.11)
						0.08 (0.07-0.10)
						0.09 (0.06-0.11)

*in millimeters of the average and the range in parentheses.

Table II. Anisakidae larvae from various fishes and squids caught in the Gulf of Tongking

Host	Number examined	Number of fishes and squids infected with <i>Anisakis simplex</i> larvae	Infection rate (%)	Anisakidae larvae of other than <i>Anisakis simplex</i>
1 <i>Priacanthus tayenus</i>	7	7	100	
2 <i>Muraenesox cinereus</i>	4	4	100	H
3 <i>Decapterus maruadsi</i>	2	2	100	
4 <i>Branchiostegus argentatus</i>	2	2	100	
5 <i>Trichiurus haumela</i>	7	6	85.7	H
6 <i>Saurida elongata</i>	7	5	71.0	H
7 <i>Epinephelus areolatus</i>	3	2	67.0	
8 <i>Lutjanus sebae</i>	1	1	100	
9 <i>Psettodes erumei</i>	2	1	50.0	H, P
10 <i>Therapon jarbua</i>	9	4	44.4	
11 <i>Lutjanus erythropterus</i>	5	2	40.0	H
12 <i>Evynnis cardinalis</i>	4	1	25.0	
13 <i>Rhabdosargus sarba</i>	4	1	25.0	
14 <i>Therapon theraps</i>	14	2	14.2	
15 <i>Trachurus japonicus</i>	9	1	11.1	
16 <i>Nemipterus virgatus</i>	9	0	0	
17 <i>Megalaspis cordyla</i>	6	0	0	
18 <i>Pneumatophorus japonicus</i>	5	0	0	
19 <i>Aluterus monoceros</i>	5	0	0	
20 <i>Ephippus orbis</i>	5	0	0	
21 <i>Lutjanus vitta</i>	4	0	0	
22 <i>Zonichthys nigrofasciata</i>	4	0	0	
23 <i>Nemipterus japonicus</i>	4	0	0	
24 <i>Ilisha elongata</i>	3	0	0	
25 <i>Rachycentron canadum</i>	2	0	0	
26 <i>Caranx chrysophry</i>	2	0	0	
27 <i>Chiloscyllium plagiosum</i>	2	0	0	
28 <i>Pomadasys hasta</i>	2	0	0	
29 <i>Polydactylus sextarius</i>	1	0	0	
30 <i>Sepia</i> sp.	3	0	0	
31 <i>Loligo formosana</i>	3	0	0	
Total	140	41	29.3	

H: *Hysterothylacium*, P: *Terranova* Cannon Type I

Table III. Measurements* of *Anisakis simplex* larvae from fishes of the East China Sea and the Yellow Sea

Host	Number of the larvae examined	Length	Width	Oesophagus			Tail
				Muscular	Glandular	Total	
<i>Pneumatophorus japonicus</i>	26	18.92 (13.21-22.47)	0.40 (0.28-0.50)	1.84 (1.28-2.10)	0.67 (0.50-0.76)	2.42 (1.88-2.80)	0.08 (0.06-0.11)
<i>Trichiurus haumela</i>	27	17.39 (12.30-21.15)	0.38 (0.26-0.50)	1.57 (1.33-1.98)	0.59 (0.47-0.69)	2.20 (2.03-2.60)	0.08 (0.06-0.11)
<i>Pseudosciaena crocea</i>	6	18.70 (17.30-21.12)	0.47 (0.46-0.50)	1.83 (1.70-1.91)	0.70 (0.61-0.79)	2.50 (2.39-2.58)	0.09 (0.09-0.11)
<i>Johnius belengerii</i>	3	17.76 (15.21-19.90)	0.44 (0.41-0.49)	1.64 (1.44-1.74)	0.70 (0.63-0.82)	2.31 (2.13-2.50)	0.10 (0.08-0.12)
<i>Muraenesox cinereus</i>	4	17.33 (17.04-17.73)	0.40 (0.40-0.41)	1.82 (1.74-1.90)	0.61 (0.50-0.71)	2.40 (2.24-2.54)	0.09 (0.09-0.10)
<i>Trachurus japonicus</i>	7	17.74 (14.93-19.51)	0.44 (0.40-0.50)	1.74 (1.52-2.00)	0.64 (0.51-0.82)	2.42 (2.20-2.71)	0.08 (0.07-0.11)
<i>Taiax tumifrons</i>	4	19.47 (17.92-22.08)	0.52 (0.43-0.60)	1.83 (1.73-1.94)	0.62 (0.58-0.70)	2.40 (2.34-2.51)	0.09 (0.07-0.11)
<i>Lophius litulon</i>	6	19.23 (17.14-22.50)	0.53 (0.44-0.60)	1.84 (1.72-2.02)	0.74 (0.64-0.84)	2.54 (2.33-2.81)	0.11 (0.09-0.12)
<i>Saurida elongata</i>	2	19.43, 20.32	0.40, 0.50	1.60, 2.20	0.71, 0.78	2.32, 2.90	0.09, 0.12
<i>Uranoscopus japonicus</i>	2	17.61, 19.54	0.41, 0.40	1.50, 1.57	0.53, 0.70	2.04, 2.31	0.08, 0.10
<i>Pseudosciaena polyactis</i>	2	18.02, 18.50	0.40, 0.40	1.51, 1.60	0.51, 0.60	2.04, 2.23	0.08, 0.09
<i>Scomberomorus niphonius</i>	2	17.92, 20.40	0.41, 0.48	1.50, 1.78	0.61, 0.68	2.22, 2.42	0.09, 0.08
<i>Chelidonichthys kumu</i>	2	16.14, 19.53	0.40, 0.41	1.50, 1.78	0.51, 0.56	2.14, 2.54	0.08, 0.09
<i>Epinephelus awoara</i>	2	16.41, 17.72	0.41, 0.50	1.70, 1.80	0.50, 0.70	2.23, 2.41	0.08, 0.09
<i>Paralichthys olivaceus</i>	2	18.14, 21.12	0.40, 0.47	1.80, 1.87	0.72, 0.78	2.61, 2.60	0.08, 0.90
Total	97	18.50 (12.30-22.50)	0.43 (0.26-0.60)	1.72 (1.28-2.20)	0.64 (0.47-0.84)	2.37 (1.88-2.90)	0.09 (0.06-0.12)

*in millimeters of the average and the range in parentheses.

Table IV. Anisakidae larvae from various fishes and squids caught in the East China Sea and the Yellow Sea

Host	Number examined	Number of fishes and squids infected with <i>Anisakis simplex</i> larvae	Infection rate (%)	Anisakidae larvae of other than <i>Anisakis simplex</i>
1 <i>Pneumatophorus japonicus</i>	33	33	100	H
2 <i>Trichiurus haumela</i>	22	22	100	H, P
3 <i>Muraenesox cinereus</i>	6	6	100	H
4 <i>Saurida elongata</i>	16	14	87.5	H
5 <i>Taius tumifrons</i>	24	21	87.5	H, R, P
6 <i>Uranoscopus japonicus</i>	7	6	85.7	H, P
7 <i>Pseudosciaena polyactis</i>	20	17	85.0	H, R
8 <i>Pseudosciaena crocea</i>	13	11	84.6	H, R, P
9 <i>Johnius belengerii</i>	12	10	83.3	H
10 <i>Lophius litulon</i>	6	5	83.3	H
11 <i>Trachurus japonicus</i>	23	19	82.6	H
12 <i>Scomberomorus niphonius</i>	23	18	78.2	H
13 <i>Ilisha elongata</i>	6	4	66.6	
14 <i>Pagrosomus major</i>	6	4	66.6	H, R
15 <i>Sebastiscus marmoratus</i>	5	3	60.0	H
16 <i>Chelidonichthys kumu</i>	6	3	50.0	H
17 <i>Branchiostegus argentatus</i>	6	3	50.0	H
18 <i>Lepidotrigla microtera</i>	6	2	33.3	H
19 <i>Paralichthys olivaceus</i>	1	1	100	
20 <i>Pseudorhombus arsius</i>	1	1	100	H
21 <i>Hoplobrotula armata</i>	1	1	100	H
22 <i>Navodon modestus</i>	4	2	50.0	
23 <i>Epinephelus awoara</i>	2	2	100	H
24 <i>Sepia</i> sp.	20	2	10.0	
25 <i>Cynoglossus robustus</i>	1	0	0	
26 <i>Pleuronichthys cornutus</i>	10	0	0	
27 <i>Nemipterus virgatus</i>	6	0	0	
28 <i>Stromateoides argenteus</i>	36	0	0	
29 <i>Raja hollandi</i>	5	0	0	
30 <i>Priacanthus macracanthus</i>	5	0	0	
31 <i>Sphyræna pinguis</i>	5	0	0	
32 <i>Katsuwonus pelamis</i>	2	0	0	
33 <i>Cleisthenes pinetorum</i>	1	0	0	
34 <i>Zebrias zebra</i>	1	0	0	
35 <i>Loligo formosana</i>	9	0	0	
Total	350	210	60.0	

H: *Hysterothylacium*, P: *Terranova* Cannon Type I,
R: *Raphidascaris* or *Raphidascaroides*

Anisakidae larvae of other than *Anisakis simplex* were also detected, such as those of several types of *Hysterothylacium* and *Terranova* (Cannon Type I) (13). The larvae of *Pseudoterranova decipiens*, pathogenic to humans, were not found in this survey.

*Anisakidae Larvae Found in Marine Fishes and Squids Captured
in the East China Sea and the Yellow Sea*

Thirty-five species of marine fishes and squids were investigated for Anisakidae larvae infection. The detected *Anisakis simplex* larvae were measured and morphologically examined (Table III). The larvae were identified in the way referred to above.

In 24 species of fishes and squids tested, *Anisakis simplex* larvae infection was confirmed (Table IV). The number of fishes and squids examined was 350, and the number of those infected with the larvae 210. The infection rate was 60.0%.

Other Anisakidae worms detected consisted of larvae belonging to several types of *Raphidascaris* or *Raphidascaroides*, *Hysterothylacium*, and *Terranova* (Cannon Type I). Even in this survey, the larvae of *Pseudoterranova decipiens* were not detected.

DISCUSSION

The Anisakidae larvae infection is commonly found among fishes and squids in seas throughout the world.

Anisakis simplex larvae are most important among such Anisakidae larvae from a standpoint of preventive medicine, as they are pathogenic to humans and very commonly found in foodstuffs such as marine fishes and squids. Many reports of *Anisakis simplex* larvae infection in marine fishes and squids have been published in the world, especially in Japan. Such studies, however, have not been published in China, except for one report by Dr. Hsu et al. and two reports by Dr. Sun et al (10-12).

The present study was carried out for the purpose of clarifying the situation of *Anisakis* infection among the seafishes and squids captured off the coast of China. As a result of this research, the following facts were made clear: (1) *Anisakis simplex* larvae were detected in many kinds of inshore and offshore fishes in China; (2) the infection rates were 29.3% in fishes and squids captured

in the Gulf of Tongking and 60.0% in those captured in the East China Sea and the Yellow Sea; (3) the infection rates showed considerably high fluctuation among fish species, and (4) the infection was found for the first time in *Sepia* sp. All of these facts were previously unknown in China.

It is not understood why the infection rate of *Anisakis* Type I larvae in fishes and squids captured in the Gulf of Tongking is lower than that in the East China Sea and the Yellow Sea. This is a very interesting problem to be solved in the future.

In the countries other than China surrounding the Yellow Sea, the East China Sea or the South China Sea, several reports concerned with *Anisakis* larvae have been published (14-17). The situations of *Anisakis* infection in marine fishes described in these reports are very similar from one to another.

Squids belonging to *Doryteuthis*, *Gonatus*, *Onychoteuthis*, *Todarodes* and others (Suborder: Teuthoidea) have been known to be intermediate hosts of *Anisakis simplex*. In the present study, the infection was confirmed even in squids belonging to *Sepia* (Suborder: Sepioidea) for the first time. The fact is very important from the food hygienic point of view, because more squid species than those known before were found to be contaminated with *Anisakis simplex* larvae.

Anisakiasis has never been found among the Chinese people. Several conditions conducive to human anisakiasis, however, are now present in China. Such conditions are the high infection rates in seafishes and squids in the seas near China, the existence of the custom of eating raw fishes and squids among some Chinese people and the recent importation of the custom of eating Sushi and Sashimi from Japan to China. In these circumstances, it is likely that human cases of anisakiasis will occur in China in the near future.

Hsu et al. (10) reported that such seafishes as *Coilia ectines* which go up a river at the egg-laying time were contaminated with *Anisakis simplex* larvae. If this is true, the Chinese people will also have to be careful of the infection through such anadromous fishes, which are also a protein source for them.

Raphidascaris or *Raphidascaroides* larvae are nonpathogenic to humans, therefore, they are not discussed further in this report. The pathogenicity of *Hysterothylacium* larvae to humans is still unclear. No human cases of parasitic diseases caused by the larvae have been reported in the world. More work on the larvae will be necessary. *Terranova* Cannon Type I larvae may belong to another genus, *Pseudoterranova*, according to Gibson's assertion (18). Though the larva of *Pseudoterranova decipiens* is known to be pathogenic to humans, it has not been clear whether *Terranova* Cannon Type I larva is pathogenic to humans or not.

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