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The infection status of anisakid larvae in marine fish and cephalopods from the Bohai Sea, China and their taxonomical consideration

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Abstract: The infection status of anisakid larvae was examined in 290 marine fish of 25 species and in 108 cephalopods of 3 species purchased in Bayuquan region, Yingko city nearby the coast of the Bohai Sea from may to August 1992. A total of 7,327 larvae were collected from 156 fish of 19 species and 8 squids of one species. The 3rd-stage larvae of *Anisakis simplex* were collected from 121 fish (63.4%) of 15 species (N = 191) and from 8 squids (14.8%) of one species (N = 54), and they were total, 5,992 (81.8%). Out of remaining 1,335 larvae, 154 (2.1%) were classified as *Thynnascaris* type B from 23 fish of 4 species, 1,013 (13.8%) as *Thynnascaris* type C from 79 fish of 13 species, 164 (2.2%) as *Hysterothylacium* China type V from 20 fish of 4 species, 3 (0.04%) as *Raphidascaris* from 3 fish of 2 species and one was *Pseudoterranova decipiens* larva.

Key words: Bohai Sea, *Anisakidae*, larvae, *Anisakis*, *Hysterothylacium*, *Thynnascaris*, *Raphidascaris*, *Pseudoterranova*.

INTRODUCTION

Anisakid nematodes such as *Anisakis*, *Pseudoterranvoa*, *Hysterothylacium*, *Raphidascaris* are parasitic in the stomach of marine mammals and/or fish-eating birds (Oshima, 1972a & b). Their larvae are encysted in marine fish or squids. It is well known that some of these larvae can cause eosinophilic granuloma at the gastrointestinal wall and elicit various clinical manifestations of acute abdomen in human. Cases of human anisakiasis were reported in many countries where marine fish is consumed raw or under improperly cooked conditions, especially in Japan, 18,984 proven cases were recorded (Ishikura, 1992). There has been no reports of the human case of anisakid infection in China in the literature concerned, it is thought mainly because that the physicians do not know anisakiasis, and which is usually be diagnosed to other diseases by mistake. While it has been known that the incidence of infected larvae in marine fish is high in the Yellow Sea, the East Sea, the Gulf of Tong King (Sun *et al.*, 1986 & 1992).

However, there is still no report about the

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infection status of anisakid larvae of the fish and cephalopods in the Bohai Sea, so an investigation was given at Bayuquan region, Yingko City, Liaoning province nearby the coast of the Bohai Sea from May to August 1992.

MATERIALS AND METHODS

Two hundreds and ninety marine fish of 25 species and 108 cephalopods were purchased from fishermen in Bayuquan region (N 40°15′, E 122°5′), Yingko city nearby the coast of the Bohai Sea from May to August, 1992. They were transported to the laboratory, the weight, length and width of the fish and cephalopods were measured and after opening their thorax and abdomen, larvae were collected from the liver, stomach, intestine, air bladder, and from their organ walls, *i.e.* the liver capsule, omentum, and anterior and posterior abdominal wall, the abdominal muscle of the hosts were digested in a solution of 0.85% HCl and 1% pepsin at 37°C for 3-4 h. The larvae were fixed and cleared with glycerine alcohol (1:9) and observed under the microscope.

RESULTS

A total of 7,327 (still another 29 could not be identified) anisakid larvae were collected from 156 fish of 19 species and 8 squids of one species, the infection rate of the fish was 53.8% and the infection density was from 1 to 929 (Table 1).

The 3rd-stage larvae of *A. simplex* were identified from 121 fish (63.4%) of 15 species (N = 191) and 8 squids (14.8%) of one species (N = 54), and their total number of larvae was 5,992 (81.8%), and the infection density was different from 1 to 880. High infection rate and infection density were shown in *Nibea albiflora* (40/50, 1~250 larvae), *Lateolabrax japonicus* (10/10, 76~880), *Odontobutis obscura* (32/35, 1~201), *Scomberomorus niphonius* (9/15, 1~74), *Trichiurus haumela* (6/6, 3~18), and *Hexagrammos otakii*. A linear correlation were noted between the parasitic number and the weight in *L. japonicus* (Y = 71.6X + 15.9, r = 0.99) and *S. niphonius* (Y = 17.2X − 5.6, r = 0.96), and also a linear correlation between the parasitic number and the length in *L. japonicus* (Y = 13.3X − 610, r = 0.95) and *S. niphonius* (Y = 1.3X − 57.9, r = 0.94) and respectively, but there was no linear correlation in *N. albiflora*. The localization of *A. simplex* larvae was different according to the kinds of fish, they were mainly in the stomach wall and mesentery, and some were in the liver, body cavity, sexual gland, intestine wall, pyloric caeum, fatty mass, air bladder and muscle (Table 2).

Among the other 1,335 larvae, 154 larvae (2.1%) were classified as *Thynnascaris* type B from 23 fish of 4 species, 1,013 larvae (13.8%) as *Thynnascaris* type C from 79 fish of 13 species, 164 larvae (2.2%) as *Hysterothylacium* China type V from 20 fish of 4 species, 3 larvae (0.04%) as *Raphidascaris* from 3 fish of 2 species and one was *Pseudoterranova decipiens* larva from a *L. japonicus*, the hosts are listed in Table 1, the dimension and index of anisakid larvae are listed in Table 3.

While there were no anisakid larvae collected from 6 species of fish and 2 species of cephalopods such as *Llisha elongata* (Number of examined = 15), *Trachurus japonicus* (N = 17), *Priacanthus macrocanthus* (N = 15), *Liza haematocheila* (N = 8), *Miichyts miiuy* (N = 1), *Pseudosciaena crocea* (N = 6); *Octopus ocellatus* (N = 38) and *Ommastrephaes sloani* (N = 18).

DISCUSSION

It is well known that there are 3 species in genus *Anisakis* such as *A. simplex* Rudolphi, 1908, *A. physeteris* Baylis, 1923 and *A. typica* Diesing, 1860. The adult of larval type I and type II are *A. simplex* and *A. physeteris*, respectively. Little is known about larval type III & IV and their adults (Otsuru *et al.*, 1969). Since the *Anisakis* larva type I has a wide suitability, 249 species of fish and squids are known to serve as the host of the larvae. In China, 56 species of fish and cephalopods were infected with the larvae in the East Sea, Yellow Sea, Gulf of Tong King, Liaohe River, Tumenjiang River and Heilongjiang River (Sun *et al.*, 1986, 1992; Hsu, 1934; Ma *et al.*, 1993). The total infection rate was high as 84%, and the infection density (number infected of larvae

Table 1. Infection status with anisakid larvae found in marine fish and cephalopods from the Bohai Sea

Host	No. of fish examined	No. of fish positive (%)	No. (%) of larvae collected Total	As[a]	TB[b]	TC[c]	HC5[d]	R[e]	Pd[f]
Fishes									
Nibea albiflora	50	45 (90)	1,716	1,105(64.4)	55(3.2)	400(23.3)	154(9.0)	2	0
Pseudosciaena polyactis	10	7 (70)	159	58(36.5)	72(45.3)	28(17.6)	0	1	0
Lateolabrax *japonicus*	10	10(100)	3,941	3,880(98.5)	0	60(1.5)	0	1	1
*Odontobut obscura**	35	32(91.4)	878	600(68.3)	12(1.4)	261(29.7)	5	0	0
Argyrosomus agentatus	25	19(76)	192	11(5.7)	0	177(92.2)	4	0	0
Scomberomorus niphonius	15	9(60)	196	196(100)	0	0	0	0	0
Trichiurus haumela	6	6(100)	49	45(91.8)	4(8.2)	0	0	0	0
Harengula zunasi	17	9(53)	12	6(50)	0	6(50)	0	0	0
Hexagrammos otakii	1	1(100)	51	26(51)	7(13.7)	18(35.3)	0	0	0
Paragrosomus major	2	2(100)	13	12(92.3)	0	1(7.7)	0	0	0
*Cynoglossus semilaevis**	7	1(14.3)	1	1(100)	0	0	0	0	0
*Platichtys bicoloratus**	2	2(100)	32	2 (6.3)	0	29(90.6)	1	0	0
Paralichtys olivaceua	2	1(50)	9	9(100)	0	0	0	0	0
Raja porosa	6	1(16.7)	1	1(100)	0	0	0	0	0
*Ablennes anstomella**	3	2(66.7)	14	2(14.3)	0	12(85.7)	0	0	0
Stromateoides argentcus	10	5(50)	7	0	0	7(100)	0	0	0
Navodon modestus	1	1(100)	4	0	4 (100)	0	0	0	0
Clupanodon punctatus	14	1(7.1)	12	0	0	12(100)	0	0	0
Cypselures agoo	12	2(16.7)	2	0	0	2(100)	0	0	0
Cephalopods									
Sepia esculenta	54	8(14.8)	38	38(100)	0	0	0	0	0

[a]*Anisakis simplex*, [b]*Thynnascris* type B; [c]*Thynnascaris* type C; [d]*Hysterothylacium* China type V; [e]*Raphidascaris* sp.; [f]*Pseudoterranova decipiens*.
*New hosts of the larval *A. simplex*.

Table 2. Localization of *A. simplex* larvae (%) collected in 4 species of fish

Localization	Number of *A. simplex* larvae (%) collected in fish S.n. (N = 15)	N.a. (N = 50)	O.o. (N = 35)	L.j. (N = 10)[a]
mesentery	85(43.4)	931(84.3)	260(43.3)	464(12.0)
stomach wall	2 (1.0)	82 (7.4)	230(38.3)	2,995(77.2)
liver	86(43.9)	29 (2.6)	1 (0.2)	27 (0.7)
body cavity	1 (0.5)	1 (0.1)	2 (0.3)	79 (2.0)
sexual gland	22(11.2)	43 (3.9)	25 (4.2)	0
intestinal wall	0	0	16 (2.7)	133 (3.4)
pyloric caecum	0	0	66(11.0)	0
fatty mass	0	0	0	87 (2.2)
air bladder	0	4 (0.4)	0	0
abdominal muscle	0	6 (0.5)	0	95 (2.4)
Total*	196 (3.3)	1,105(18.6)	660(10.1)	3,880(65.2)

*Compared with the total *A. simplex* larvae (5,954) collected in fish
[a]*S.n: S. niphonius; N.a: N. albiflora; O.o: O. obscura; L.j: L. japonicus*

Table 3. Dimension[a)] and index of anisakid larvae found in several species of marine fish and cephalopods from the Bohai Sea

larvae[b)] / Item	As	HC5	R	Pd	TB female	TB male	TC female	TC male
No. specimens examined	69	16	3	1	5	4	12	8
Body length	13.5-37.0	12.3-19.5	8.5-9.9	40.0	13.0-23.4	10.0-24.0	9.2-23.5	11.0-16.0
	22.5	16.1	9.4		17.8	16.9	13.7	13.4
Body width	0.26-0.74	0.37-0.63	0.24-0.27	0.87	0.21-0.33	0.17-0.37	0.16-0.46	0.18-0.29
	0.50	0.48	0.27		0.27	0.26	0.24	0.20
Esophagus	2.07-3.81	1.11-1.49	0.86-1.01	3.51	1.77-2.45	1.00-2.54	1.28-2.76	1.64-2.20
Total	2.76	1.35	0.95		2.21	1.96	1.77	1.77
Muscular	1.54-2.56	1.05-1.36	0.80-0.90	1.20	0.15-0.20	0.08-1.18	0.06-0.20	0.08-0.15
	1.99	1.24	0.87		2.04	1.83	1.65	1.66
Ventriculus	0.51-1.25	0.10-0.15	0.06-0.11	1.20	0.15-0.20	0.08-1.18	0.06-0.20	0.08-0.15
	0.74	0.12	0.08		0.17	0.12	0.12	0.11
Ventricular appendix		3.75-5.51	0.47-0.55		0.83-1.34	0.92-1.63	1.06-1.73	1.0201.47
		4.85	0.52		1.22	1.31	1.37	1.17
Intestinal caecum		0.16-0.21		0.82	0.83-1.60	0.58-1.47	0.74-1.66	0.79-1.06
		0.18			1.19	1.14	1.07	0.99
Tail	0.08-0.15	0.14-0.20	0.11	0.18	0.19-0.30	0.07-0.17	0.06-0.24	0.06-0.13
	0.12	0.17	0.11		0.22	0.13	0.17	0.09
BL/BW[c)]	33.5-60.3	27.4-38.1	34.6-35.4	46.0	57.1-84.0	58.3-71.4	50.9-68.8	47.5-66.7
	45.6	34.0	35.1		67.7	64.1	59.2	59.5
BL/Es	6.4-11.1	10.7-13.2	9.9-10.1	11.4	6.5-10.0	7.4-10.0	6.5-8.6	5.7-9.4
	8.2	11.9	10.0		8.1	8.8	7.7	7.7
BL/M	8.0-16.2	11.7-13.2	10.6-11.1	17.4	6.9-10.9	7.8-10.9	7.2-9.2	6.0-10.2
	11.3	13.0	10.8		8.7	9.4	8.2	8.2
BL/V	22.1-35.7	94.3-172.5	110.0-141.7	33.3	74.3-146.3	133.3-165.0	71.8-230.6	96.0-162.5
	29.7	134.7	124.4		107.1	146.3	118.8	124.4
BL/T	103.5-336.3	82.9-107.1	65.4-90.0	228.6	65.0-123.2	100.0-191.8	94.0-129.6	104.5-213.0
	198.1	95.4	78.6		82.4	140.4	82.0	156.6
BL/VA		2.8-4.5	17.6-18.1		11.5-28.2	9.3-28.2	8.0-13.6	8.8-17.3
		3.4	17.9		17.0	14.3	10.0	11.9
BL/IC		72.4-107.0		48.8	10.0-20.5	11.2-25.0	10.4-15.2	11.7-16.4
		88.4			15.7	16.3	12.7	13.5
VA/IC		19.5-31.5			0.8-1.2	0.9-1.6	1.0-1.8	0.84-1.44
		26.7			0.9	1.23	1.3	1.1
V/IC				1.5				
Boring tooth	+	+	+	+	−	−	+	+
Interlabia	−	+	−	−	+	+	+	+
Excretory pore	between subventral lips	behind nerve-ring		behind sub-ventral lips	behind	nerve		ring
Genital anlage	−	−	+	−	+	+	+	+
Mucron	+	+	+	+	−	−	+	+

a)Unit is mm; b)As, *Anisakis simplex;* TB, *Thynnascris* type B; CT, *Thynnascaris* type C; HC5, *Hysterothylacium* China type V; R, *Raphidascaris* sp.; Pd, *Pseudoterranova decipiens.* c)BL, body length; BW, body width; Es, esophagus; M, muscular part of esophagus; V, ventriculus; T, tail; VA, ventricular appendix; IC, intestinal caecum.

per specimen) was heavy in 9 species of fish including *Pneumatophorus japonicus* (1~437 larvae), *T. haumela* (18~102), *P. polyactis* (1~82) and *P. crocea* (3~122) (Sun *et al.*, 1986). A similar infection rate and infection density was found in our investigation of larval *A. simplex*. *A. simplex* larvae were most frequent (5,992 in number, 81.8%), the infection rate of the fish was 63.4%, and the infection density was from 1 to 880. A linear correlation was noted between the parasite number and the weight or the length of *L. japonicus* and *S. niphonius*, respectively. This means that the longer and heavier the fish are, the more the *A. simplex* larvae infect. However, no correlation was found in *N. albiflora*. Larval *A. simplex* were found in the muscle of *Pneumatophorus japonicus* and *T. haumela* (Sun *et al.*, 1986), while we did not collect the larvae in the muscle of *T. haumela*. In our study, the larvae were collected in the abdominal muscle of *L. japonicus* and *N. albiflora*, so the two species of fish are regarded important as possible source of anisakiasis in China.

It should be noticed that some species of fish, such as *T. japonicus*, *Navodon modestus*, *P. crocea* and *Llisha elongata*, were infected nearby the coast of the East Sea and the Yellow Sea (Sun *et al.*, 1986 & 1992), whereas not in our investigation, it was probably according to the difference of food chains, investigation season and span, number of the fish examined and the different place of the investigation. Though it was negative for these species of fish, should it not keep out the possibility of infection because of the little number of fish examined.

Among these species of fish, *Platichtys bicoloratus*, *Cynoglossus semilaevis*, *Ablennes anstommella* and *O. obscura* were the new hosts of *A. simplex* larvae.

The taxonomy of Anisakidae larvae is confused, especially in the genus *Contracaecum*, as many as 20 or more larval types have been reported (Yamaguti, 1935 & 1941; Koyama *et al.*, 1969; Kagei *et al.*, 1979; Kikuchi *et al.*, 1970; Deardorff & Overstreet, 1980, 1981a & 1981b; Smith, 1983; Chai *et al.*, 1986). Koyama *et al.* (1974) renamed *Contracaecum* type A, C, D as *Thynnascaris* larval type A, B, C, respectively. We still adopt the name of *Thynnascaris* in this paper, and we also adopt larval *A. simplex* (= *A.* type I) [*Anisakis* Dujardin, 1845 — type I according to Berland, 1961], larval *Pseudoterranova decipiens* (= *Terranova* type A according to Koyama *et al.*, 1969), and larval *Hysterothylacium* sp. China type V of Sun *et al.* 1992.

Besides *Anisakis* larvae, five types of larvae were recovered, such as *Thynnascaris* type B & C, *Pseudoterranova decipiens*, *Hysterothylacium* type V and *Raphidascaris* sp. Among these, 1,013 (13.8%) *Thynnascaris* type C larvae which were not easy to find were recovered from 79 fish of 13 species. This type of larvae was most frequent, and this fact should represent marine ecology in the Bohai Sea.

Hysterothylacium China type V larvae have a boring tooth and a tiny mucron, interlabia, but no genital anlage. The excretory pore opened behind nerve ring, tail ensheathed, long and slender in shape. The ratio of ventricular appendage to intestinal caecum is 26.7, which is smaller than that of Sun *et al.* (1982). The intestine was full with brown-yellow grains and the larvae emerged as brown-yellow.

Only one larval *Pseudoterranova decipiens* was found in our observation, so it is not important in the epidemic of anisakiasis in the Bohai Sea.

In conclusion, *A. simplex* larvae were actively prevalent among fish and cephalopods in the Bohai Sea. Fifteen out of 25 species of fish were infected, and 4 species were newly recognized. *Thynnascaris* sp. larvae type B & C of Koyama *et al.* (1974) are newly recorded in China. The larva of *Thynnascaris* sp. type C was recovered most frequently by infection rate and most heavily by infection density. For transmission of human anisakiasis, *N. albiflora*, *P. polyactis*, *L. japonicus*, *O. obscura*, *S. niphonius* and *T. haumel* were found inportant. Since larvae of *A. simplex* were collected from the viscera or the abdominal muscle of *N. albiflora* and *L. japonicus* and some people prefer eating raw or improperly cooked fish, these two species may be major source of human infection.

REFERENCES

Chai JY, Chu YM, Sohn WM, Lee SH (1986) Larval anisakids collected from the yellow corvina in Korean. *Korean J Parasitol* **24:** 1-11.

Deardorff TL, Overstreet RM (1980) Taxonomy and biology of North American species of *Goezia* from fishes. *Proc Helminthol Soc Washington* **48:** 113-126.

Deardorff TL, Overstreet RM (1981a) Review of *Hysterothylacium* and *Iheringascaris* from the Northern Gulf of Mexico. *Proc Bio Soc Washington* **93**(4): 1035-1079.

Deardorff TL, Overstreet RM (1981b) Larval *Hysterothylacium* from fishes and invertebrates in the Gulf of Mexico. *Proc Helminthol Soc Washington* **48:** 113-126.

Hsu HF (1934) On some *Kaliocephalus* species from China with a discussion of certain systematic characters of the genus. *Pek Nat Hist Bull* **8**(3): 375.

Ishikura H (1992) Multigenerous migration of *Anisakidae* larvae into the human bodies (5) especially on the morphology and histopathology of *Hysterothylacium aduncum. Jpn J Parasitol* **41**(1): 64.

Kagei N, Sakaguchi Y, Katamine D, Ikeda Y (1970) Studies on the anisakid nematoda II. *Contracaecum* sp. found in marine fishes. *Bull Inst Publ Health* **19**(4): 243-251.

Kikuchi S, Kosugi H, Hirabayashi H, Hayashi S (1970) Six type of *Contracaecum* larvae found in the sea fishes in Japan. *Yokohama Igaku* **21:** 421-427.

Koyama T, Kobayashi A, Kumada M, *et al.* (1969) Morphological and taxonomic studies on Anisakidae larvae found in marine fishes and squids. *Jpn J Parasistol* **18:** 466-487.

Koyama T (1974) Morphological and taxonomic studies on Anišakidae larvae found in marine fishes. Anisakiasis in fish. p. 9-19 Jpn Fishery Soc.

Ma HW, Jiang TJ, Quan FS, Lei S (1993) The preliminary investigation of *Anisakis* larvae in *Oncorhynchus keti* of Tumenjiang River. *J Yanbian Med Coll* **16**(1): 29-32.

Oshima T (1972a) Progress of medical parasitology in Japan. Tokyo: Meguro parasitological Museum **4:** 301-393.

Oshima T (1972b) Studies on the first intermediate hosts of *Anisakis. Saishin Igaku* **24:** 401-404.

Otsuru M (1969) On the morphological classification and experimental infection of Anisakidae found in marine fishes around the northern sea of Japan. *Jpn J Parasitol* **18:** 417.

Smith JW (1983) *Anisakis simplex:* morphology and morphometry of larvae from euphausiids and fish and a review of life history and ecology. *J Helminthol* **57:** 205.

Sun SZ, Zhang YL, Pan GF, Sun M (1986) Preliminary investigation on *Anisakis* larvae infestation in marine fishes. *J Parasitol Parasitic Dis of China* **4**(3): 181-183.

Sun SZ, Koyama T, Kagei N (1992) Morphological and taxonomical studies on Anisakidae found in marine fishes of China II. Gulf of Tong. *J Parasitol Parasitic Dis of China* **10**(2): 108-112.

Yamaguti S (1935) Studies on the helminth fauna of Japan 9. Nematodes of fishes. I. *Jpn J Zool* **6:** 337-386.

Yamaguti S (1941) Studies on the helminth fauna of Japan 9. Nematodes of fishes. II. *Jpn J Zool* **9:** 343-396.