[Jap. J. Parasit., Vol. 31, No. 2, 131-134, April, 1982]

Research Note

In Vitro Cultivation of *Anisakis* Type I and Type II Larvae Collected from Fishes Caught in Japanese Coastal Waters and Their Identification

TOMOO OSHIMA*, SACHIKO OYA† AND RHYOKO WAKAI*

(Received for publication; November 25, 1981)

Key words: *Anisakis, in vitro cultivation,* identification

So-called *Anisakis* Type I and Type II larvae were collected from common mackerels, *Pneumatophorus japonicus japonicus,* and alaska pollacks, *Theragra chalcogramma,* caught in the Japanese coastal waters and kept in saline, with Kanamycin sulphate (0.5 mg/ml) at 5 C over-night and transferred in cultivation medium. All the cultivation experiments were carried out at 35 C. The cultivation method deviced by Van Banning (1976) and the results of ecdysis experiments (Sommerville and Davey, 1976) of *Anisakis* Type I larvae were followed or partly modified. The first moulting of Type I larva from 3rd stage to 4th stage occured easily under 5% CO_2 and 95% air in the medium TC 199 (Nissui Seiyaku Co. LTD) at pH 7.0 which was changed 2–3 days between 3rd to 7th day of cultivation, while the larva cultured in bovine liver extract (BLE) at pH 2.0 under air showed less moulting rate even after 8 days of cultivation (Fig. 1).

Fresh 4th stage larvae were collected and transferred one by one in tubes containing 2 ml of solution consisted of 1 part medium TC 199 and 3 parts BLE which was adjusted at pH 4.5 and changed daily and one or two drops of fresh bovine blood were added in tube daily till the next ecdysis took place. In this way more than 30% of 4th stage Type I larvae could advance to 5th stage preadults between 7th to 14th day of second cultivation. After second ecdysis of larva the volume of bovine blood was increased to 0.5 ml per 1 worm per day. Larvae became adults after 22th day and 32 adult worms were collected on 29th day, reared from 140 4th stage larvae (Fig. 2). Those larvae kept in the media without TC 199 or lower pH of 2.0 did not develope to adult or developed to adult more poorly.

The mouth part of adult worm was surrounded by three lipps, one was large dorsal lip and the other two were small ventro-lateral lips. Two labial papillae were found on the dorsal lip and one papilla on each of ventro-lateral lipps. Each lip had anteriorly directed two lobes with dentigerous ridges. No interlabia was noted (Fig. 3). In all male specimens, 4 pairs of postanal papillae located near the end of tail and 2 pairs of adanal papillae, one of which was double papilla, were noted (Fig. 4). These arrangement of papillae was the same with those of *Anisakis simplex* and different from those of *A. typica* or

* *Dept. Parasit. School of Medicine, Yokohama City University. Urafunecho, Minami-ku, Yokohama City Japan.*

† *Kanagawa Prefectural School of Medical Technology. Nakaocho, Asahi-ku, Yokohama City Japan.*

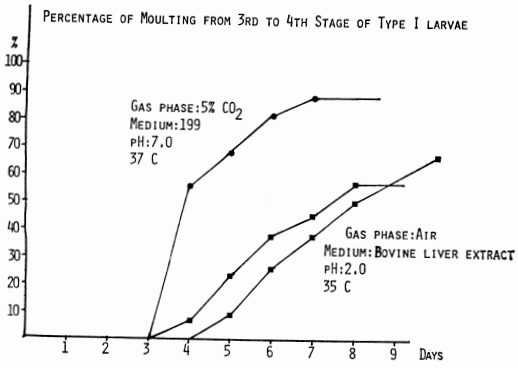

Fig. 1 Influence of gas phase on the 1st ecdysis of Type I larvae *in vitro*.

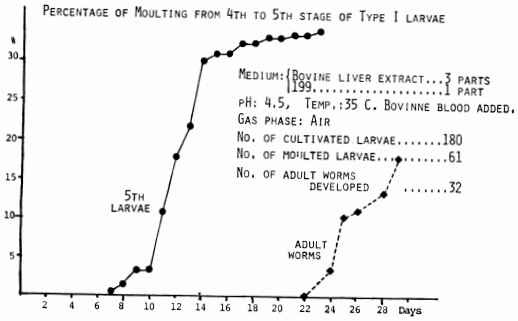

Fig. 2 Development of preadult and adult worms of Type I larvae *in vitro*.

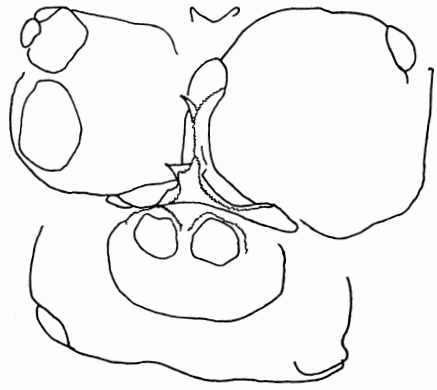

Fig. 3 Cephalic part of adult worm developed from Type I larvae *in vitro*.

A. physeteris. In Table I the lengthes and left/right ratios of spicules of 11 males were depicted. These data were almost

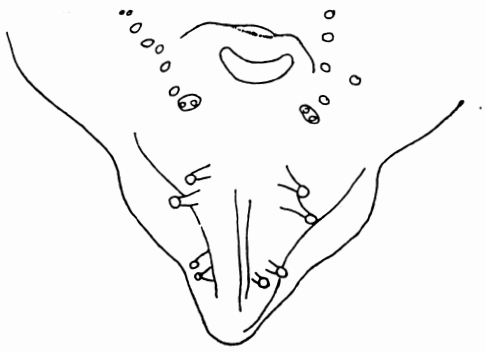

Fig. 4 Arrangement of post anal papillae.

Table 1 Lengthes and L/R ratios of adult male worms developed from Type I larvas *in vitro*.

Fish	Body length	Left spicul	Right spicul	L/R ratio
Alaska pollack	108 mm	2.6 mm	2.3 mm	1.13
	108	2.5	2.2	1.14
	88	1.8	1.6	1.22
	82	2.6	1.6	1.62
	77	2.1	1.7	1.23
	61	1.8	1.4	1.28
	56	2.1	1.4	1.50
Common mackerel	105	3.0	2.2	1.30
	65	2.4	1.6	1.50
	62	2.0	1.3	1.53
	59	1.8	1.3	1.38

Criterions:

A. simplex R/L ratio 1.17-2.35
A. physeteris, short spicul
A. typica R/L ratio 2.58-4.25
(less than 0.4 mm)

within the ranges of the length and ratio of those of *A. simplex* and clearly differentiated from those of *A. typica* and *A. physeteris* (Fig. 5).

Consequently our *Anisakis* Type I larvae were the larvae of *Anisakis simplex* as in the results of Pippy and Van Banning (1975) and Gravda (1976) who tried the cultivation of *Anisakis* Type I larvae collected from fishes caught in North Atlantic Ocean and Pomeranian Bay.

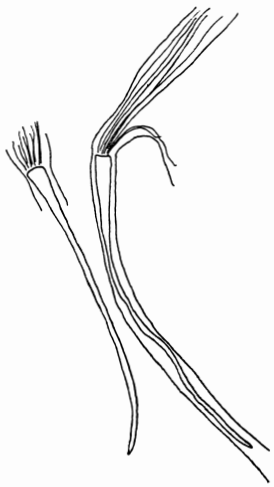

Fig. 5 Spicules of adult male worm developed from Type I larvae *in vitro*.

We Collected 12 *Anisakis* Type II larvae and tried to cultivate them in the same ways as *Anisakis* Type I larvae. But in medium TC 199 under 5% CO_2 and 95% air only 7 larvae could moult to 4th stage between 10 to 14 day of cultivation and finally 3 of them were able to moult to preadult in TC 199 and BLE medium enriched by bovine blood, however, all of them died out before 28th day and no adult worm was harvested. So-called *Anisakis* Type II larvae showed quite different attitudes by *in vitro* cultivation comparing with Type I larvae. Formerly Type II larva was suspected as the larva of *Anisakis physeteris* from it's morphological similarities of tapered tail and short venticulus (Kagei 1969).

Difficulties to cultivat Type II larvae with the same media and method used by Type I larvae suggested the posibility that the Type II larva was not the larva of marine mammal's *Anisakis*. Genus *Paranisakiopsis* (Yamaguti) was *Anisakis* of marine teleost and distinguished by prominent interlabia by adult and different figure of ventriculus in 3rd stage larva from Type II larva, however, according to Sprent (personal contact) some 3rd stage larva of this genus was almost difficult to differentiate from Type II larva. Unfortunately our cultivated Type II larvae were so weak and degenerated that we could not observe their interlabia.

As a whole, we must be cautious about the final identification of Type II larvae until we succeed their *in vitro* cultivation to adult. The authors expressed deep gratitudes to his kind advice of Dr. J.F.A. Sprent.

References

1) Banning, P. Van (1971): Some note on a successful rearing of the herring-worm, *Anisakis marina* L. (Nematoda: Heterocheilidae). J. Cons. Int. Explor. Mer., 34, 84–88.
2) Berland, B. (1961): Nematodes from some Norwegian marine fishes. Sarsia., 21–50.
3) Gravda, J. (1976): Studies on the life cycle and morphologynesis of *Anisakis simplex* (Rudolphi, 1809) (Nematodae: *Anisakidae*) cultured *in vitro*. Acta Ichthyologica et Piscatoria, 6, 119–141.
4) Kagei, N. (1969): Life cycle of *Anisakis*. Saishin Igaku, 24, 389–400.
5) Pippy, J. H. C. and van Banning, P. (1975): Identification of *Anisakis simplex* (Rudolphi, 1809) det. Krabbe 1878) Nematoda: Ascaridata). Jour. Fish. Res. Board Can., 32, 29–32.
6) Sommerville, R. I. and Davey, K. G. (1976): Stimuri for cuticule formation and ecdysis *in vitro* of the infective larvae of *Anisakis* sp. (Nematoda: Ascaridoidea). Internat. J. Parasit., 6, 433–439.
7) Yamaguti, S. (1941): Studies on the helminth fauna of Japan. Part 33. Nematodes of fishes, II. Jap. Jour. Zool., 9, 344–396.

短　　報

日本近海産魚類より採取した *Anisakis* I型ならびにII型幼虫の *in vitro* 飼育と成虫による同定について

大島智夫*　大屋幸子†　若井良子*
（横浜市立大学医学部寄生虫学教室*，神奈川県立衛生短大†）

日本近海で捕獲されたヒラサバ，スケトウダラより分離した *Anisakis* I型およびII型を試験管内で発育せしめ成虫を得ることを試みた．

I型は5% CO_2 95%空気相の下，pH 7.0，35C のTC199中で3日より7日の間にほとんど脱皮第IV期にすすみ，さらに TC199 の1容と牛肝ペプシン消化液容の pH 4.5の液に新鮮牛血液を加え 35C の培養液中で12～22日間に1/3は第5期に進み，28日までに1/6は成虫にまで発育し，成虫の形態を精査したところ *Anisakis simplex* であった．

II型はI型と同様の培養法では成虫を得られず，培養中に死滅した．II型は形態的に *Anisakis physeteris* に接続するとされていたが，果して哺乳類の *Anisakis* の幼虫であるかは慎重な検討を要し，魚類寄生のものの幼虫である可能性もある．