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Identification of Anisakis larva (I) as Anisakis simplex (Rud., 1809, det. Krabbe, 1878) (Nematoda: Ascaridata)

by

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ABSTRACT

Adult parasitic nematodes, previously reared in vitro from Anisakis larvae (I) from herring, mackerel and Norway haddock from the North Sea, are compared with recent descriptions of valid species and identified as Anisakis simplex (Rud., 1809, det Krabbe, 1878).

INTRODUCTION

Larval nematodes belonging to the genus Anisakis Dujardjn, 1845, have been described from a variety of marine and anadromous teleosts since the early 1800's. These larvae have been described under a variety of taxa, many of which were undoubtedly polyphyletic. Notable among the more recent authors who have attempted to clarify the taxonomy of the larvae were Punt (1941), Baylis (1944), Johnston and Mawson (1945) and Dollfus (1953). However, it was not until Crainger (1959) induced the larvae to moult to the pre-adult stage that they were positively identified as larvae belonging to the genus Anisakis. Subsequently, Berland (1961) identified two types of Anisakis larvae: the common form, which he called Anisakis larva (I), and an unusual form from Lampris guttatus which he called Anisakis larva (II). Larva (II) had a shorter ventriculus than did larva (I) and it did not possess a tail spine. Berland noted that the two types of larvae probably belonged to separate species. Punt (1941) suggested that only one species was represented among his collections of larvae (Anisakis larva I) and several authors (e.g. Yamaguti (1935) and Crainger (1959)) have expressed the opinion that the common larvae were Anisakis simplex. However, positive identification of the larvae could not be made until they were reared to the adult stage in the laboratory and compared with descriptions of recognized species.

Van Banning (1971) successfully reared larvae from herring, mackerel, and Norway haddock. However, he did not study the morphology of the adults he cultured and simply referred to them as Anisakis marina. This identification was based on the proposal by van Thiel (1966) that all adult species of Anisakis in sea mammals in the North Sea and South Atlantic belong to the same species, A. marina, and that the specific name for the larvae in herring should therefore be A. marina. However, Khalil (1969) rejected van Thiel's proposal because he did not provide any evidence in support of his view. Davey (1971) recognized three valid species of Anisakis, (A. simplex (Rudolphi, 1809, det. Krabbe, 1878), A. typica (Diesing, 1860) and A. physeteris (Baylis, 1923)) and rejected van Thiel's "marina" because it depended on acceptance of Gordius marinus of Linnaeus 1767, the description of which Baylis stated was inadequate. Van Banning's (1971) study could not, therefore, be considered as having solved the problem of the identity of the larvae. The purpose of this

study was to (1) determine the type of larvae used by van Banning and (2) present a specific identification of the adults reared by van Banning.

Human anisakiasis was first found in Holland in 1955 (Kuipers et al, 1960a; Kuipers et al, 1960b; v. Thiel et al, 1960) and was related to consumption of improperly prepared infected herring. From 1955 to 1967, 149 proven cases had been reported from the Netherlands (Polak and Kampelmacher, 1966; Bijkerk, 1969). Similar cases have been identified in Norway (Davey, 1972) and several hundred cases have been reported from Japan (Okierura, 1967). The public health aspects of the larvae in herring have been reviewed by Davey (1972). Larvae involved in human anisakiasis have been identified with Berland's Anisakis larva (I) (Okierura, 1967). Specific identification of the larvae involved will enable consideration of human anisakiasis with respect to the marine mammals harbouring the adult population of the species.

METHODS

Methods of collection and rearing of the larvae in vitro are given by van Banning (1971). Larvae from herring were selected for morphological and morphometric examination; these were frozen while in the culture medium, thawed, examined and photographed, and measurements were obtained from projected images of the negatives. Adults from van Banning's experiments were selected on the basis of condition and visability of taxonomically important features. They were fixed in 70% ethanol, and cleared in lactophenol or pure glycerine before examination. Taxonomically important structures in the adults were drawn with the aid of a camera lucida and measurements obtained from the drawings. The location of the vulva in the females was determined by dissection. En face views were prepared in glycerine jelly.

RESULTS AND DISCUSSION

The larvae

Morphology and morphometry of the larvae (Table 1) conformed with data given by Punt (1941) for Anisakis sp. larvae from thirteen species of marine teleosts. The shape of the ventriculus and the presence of a tail spine conformed with Berland's Anisakis larva (I).

The adults

The following observations were based on examinations of five male and five female adult specimens reared in vitro by van Banning (1971) from Anisakis larvae (I).

Spicules: These were unequal, the right being shorter than the left. The ratio of right to left spicule was 1:1.15 to 1:1.32 (Table 2). With the exception of the ratio for the specimen with a ratio of 1:1.15, the ratios conformed with the range sited by Davey (1971) for A. simplex (1:1.17 to 1:2.35). The ratio for the unusual specimen was much lower than that sited for A. typica (1:2.58 to 1:4.25) and intermediate between those sited for A. simplex and A. physeteris (1:1.12). However, it is unlikely that this specimen was A. physeteris because the lengths of the spicules (1.38 and 1.59 mm) were much greater than the maximum size sited for A. physeteris (0.4 mm). Generally, spicule lengths (0.51-2.01 mm) were lower than the limits given by Davey for A. simplex (1.25-3.75 mm). Davey noted that in A. simplex, larger specimens tended to have larger spicules. Since most of our specimens were smaller than those of A. simplex examined by Davey, their shorter spicules do not preclude identification as A. simplex (Fig. 1).

Postanal Papillae: The number and arrangements of the postanal papillae conformed with the number and variations in arrangements sited by Davey for A. simplex. The arrangement of the papillae of most specimens was similar to that shown in Davey's Fig. 1a.

Lips: The lips conformed with Davey's Fig. 2b of the lips of the type specimen of A. kükenthalii (= A. simplex). Davey noted that his figure illustrated the usual appearance of the lips applicable with only minor variations to all three valid species.

Ventriculus: The ventriculus of specimens less than 25 mm long was usually, but not always, slightly sigmoid. Those of larger, more mature specimens were invariably folded upon themselves so that they were very similar to the form shown in Davey's Fig. 3c (of A. typica). Davey noted that the form of the ventriculus of A. typica was not significantly different from that of A. simplex.

Vulva: The vulva was invariably close to the midpoint of the body. In this respect the specimens were similar to A. simplex and A. typica but different from A. physeteris in which the vulva opens in the first third of the body.

Tail Spine: This structure was not present in either the extended or retracted state on any of the specimens. Although the presence or absence of this structure has been used to distinguish different types of Anisakis larvae (Berland, 1969), Davey did not regard it as a reliable character in adults.

CONCLUSION

The size ratios of the spicules and the number and arrangement of the postanal papillae of the male specimens reared by van Banning (1971) from Anisakis larvae (I) indicate that van Banning's specimens were A. simplex. The authors conclude that Anisakis larvae (I) in fish in the North Sea belong to the species A. simplex. If Anisakis larva (I) represents one and only one of the three recognized species of Anisakis, all reports of these larvae in marine and anadromous fishes refer to A. simplex. In this case, the nematode responsible for most, if not all, human infections would be A. simplex.

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Table 1. Summary of morphometric data (in mm) of Anisakis larva infecting Atlantic herring from the North Sea, 1969. N is the number of observations; R is the range; $\bar{x}$ is the arithmetic mean; SD is the standard deviation; CV is the coefficient of variation expressed as %.

	N	R	$\bar{x}$	SD	CV
Total length	23	16.11-22.49	19.69	2.32	11.78
Diameter*	22	0.42- 0.56	0.49	0.03	6.12
Anterior end to nerve ring	25	0.120-0.379	0.315	0.058	18.41
Length of esophagus	20	1.81- 3.97	2.70	0.43	15.93
Length of ventriculus	20	0.58- 0.98	0.78	0.13	16.67
Width of ventriculus**	21	0.089-0.304	0.204	0.050	24.51
Length of tail***	24	0.100-0.201	0.136	0.028	20.59
Total length / Diameter	21	32.15-53.55	40.39	7.83	19.39
Total length / Length of esophagus	19	6.12-12.46	7.51	1.36	18.16
Total length / Length of tail	23	96.26-203.82	150.40	31.37	20.86

* Determined at midpoint of body.
** Determined at midpoint of ventriculus.
*** Length of tail determined from a lateral viewpoint; measurements were made from a curved line following the cuticle from the tip of the tail (excluding the tail spine) to the anterior tip of the anus.

Table 2. Spicule measurements of five specimens of Anisakis reared in vitro by van Banning (1971).

Length of worm	Length of spicule		Ratio
	Right	Left	
20 mm	0.51 mm	0.66 mm	1:1.29
28	-	1.37	-
34	.93	1.23	1:1.32
45	1.38	1.59	1:1.15
55	1.54	2.01	1:1.31

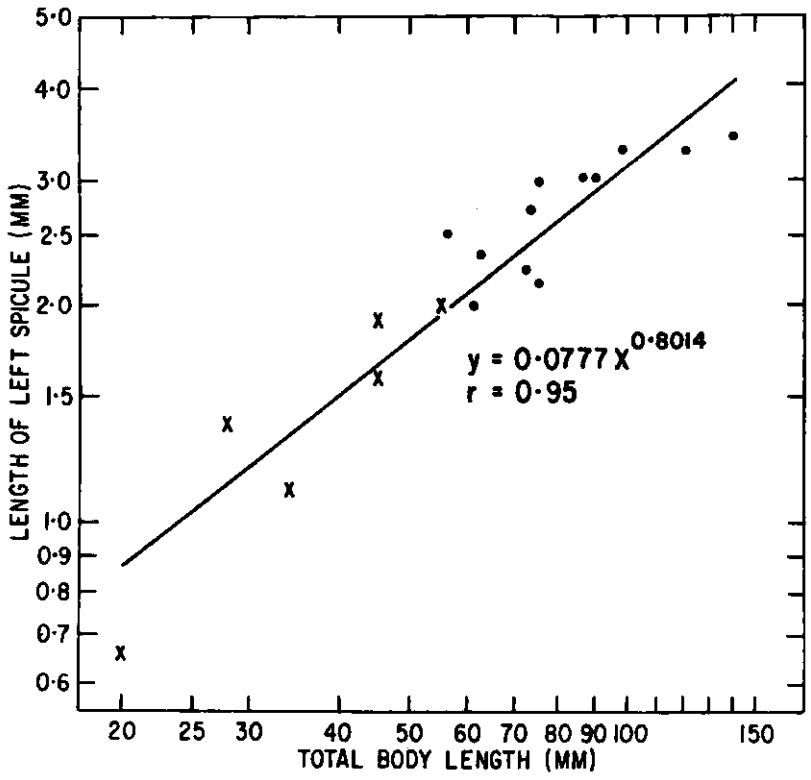

Fig. 1. Relationship of the length of the left spicule to the body length of the worm. X Specimens reared in vitro (Table 1). ● Specimens of Anisakis simplex from a single host, Orcinus orca (data from Davey, 1971).