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STICKLEBACK AS A POTENTIAL PARATENIC HOST IN THE *ANISAKIS SIMPLEX* LIFE CYCLE IN THE BALTIC SEA: RESULTS OF EXPERIMENTAL INFECTION

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CIERNIK JAKO POTENCJALNY ŻYWICIEL PARATENICZNY W CYKLU ŻYCIOWYM *ANISAKIS SIMPLEX* W BAŁTYKU NA PODSTAWIE EKSPERYMENTALNEGO ZARAŻENIA

A b s t r a c t. *Anisakis simplex* stage 3 larvae found in herring were used to experimentally infect 155 sticklebacks and 6 nine-spined sticklebacks, caught in the Gdynia marina (Gulf of Gdańsk). The larvae were observed in the fish body cavity as early as 24 h after infection, only the sticklebacks being affected. Altogether, 50 live and 2 dead larvae were found in the stickleback body cavity. The infection prevalence and mean intensity were 13.6% and 2.4 larvae, respectively, from 1 to 8 larvae per fish being recorded. It is concluded that the stickleback may serve as a paratenic host for *Anisakis simplex*.

INTRODUCTION

Anisakis simplex stage 3 larvae are common in numerous marine fish species world-wide. The nematode's presence in the Baltic Sea was first reported on in the 1960's and 1970s (LUBIENIECKI 1972, ROKICKI 1972, 1973). Occasionally, stage 3 larvae of the *Anisakis* nematodes have been found in the stickleback (SHULMANN and SHULMAN-ALBOVA 1953, POLYANSKII 1966, PODOLSKA and MOROZIŃSKA 1994) which, should the fish be a paratenic host, may enhance the chances of the parasite for closing its life cycle. Successful experimental infection of stickleback with the stage 3 larvae of *A.simplex* had already been reported (JANC and ROKICKI 1999, KOIE 2001), the experiments involving, however, low numbers of fish. The intention of the experiment, described in this paper, was to provide evidence that stickleback could, indeed, serve as a paratenic host for *Anisakis simplex*.

MATERIALS AND METHODS

The experiment involved 155 and 6 individuals of stickleback (*Gasterosteus aculeatus*) and nine-spined stickleback (*Pungitius pungitius*), respectively. The fish, caught with a dip net in the Gdynia marina, were transported to the laboratory and placed in aquaria filled with 7 ‰ seawater kept at 5-10°C and aerated. The sticklebacks were kept in 9 aquaria holding 15-20 individuals each; the nine-spined sticklebacks were kept in a separate aquarium.

The *Anisakis simplex* stage 3 larvae used to infect the stickleback were dissected out from the herring. Until used, the parasites were kept in 7 ‰ penicillin-enriched seawater. Infection was effected through a one-time release of 32-60 larvae into 7 aquaria with the sticklebacks and into the single aquarium holding the nine-spined sticklebacks. The control consisted of a total of 30 sticklebacks placed in two aquaria. The fish were sacrificed after 1, 7, 21, and 30 days from infection; the body cavity and its organs as well as the stomach and intestine were examined for the presence of the nematodes.

RESULTS AND DISCUSSION

The larvae were found in the sticklebacks only. No larvae were present in the control fish. As early as 24 h after the nematode had been released into the aquaria, the larvae were present in the fish body cavity (tab. 1).

TABLE 1
The presence of *Anisakis simplex* stage 3 larvae in the stickleback body cavity: progress of infection

No. of days after infection	No. of fish examined	No. of fish infected	No. of *A.simplex* larvae found	
			live	dead
1	3 sticklebacks	2	6	0
7	39 sticklebacks + 6 nine-spined sticklebacks + 30 control sticklebacks	10 sticklebacks infected; control sticklebacks parasite-free	22	2
14	30 sticklebacks	2	5	0
28	53 sticklebacks	8	17	0
Total	161	22	50	2

A total of 50 live larvae were found in 13.5% of the fish examined. In addition to the body cavity, the live larvae were present also in the stomach and in the gills, the latter finding being a happenstance. In addition to two dead larvae found in the body cavity, the other dead larvae and their partially digested remains were recorded in the stomach. Except for a single, 34 mm long, stickleback, the remaining infected fish measured 51-68 mm (tab. 2). Among the larvae present in the body cavity, one only was encysted; it was found in a stickleback sectioned 7 days after infection.

TABLE 2
Stickleback infection with live and dead *Anisakis simplex* stage 3 larvae by location in host. Information on live larvae present in the fish body cavity is printed with bold letters

Anisakis simplex	No. of fish infected	Fish length range [mm]	No.of parasites	Prevalence [%]	Mean intensity [ind.]	Intensity range [ind.]
Live, stomach only	2	68	3	1.3	1.5	1-2
Live, on viscera only	17	51-67	41	11	2.4	1-8
Dead + fragments in stomach	14	34-68	39+ fragments	9.1	2.8*	1-8*
Dead, on viscera only	1	59	2	0.6	2	2
Live on viscera + live in stomach	2	54-58	3on viscera + 7 in stomach	1.3	5	4-6
Liveon viscera+ deadand fragments in stomach	2	58-61	6 on viscera + 1and fragments in stomach	1.3	3*	2-5*
Remains only in stomach	3	51-61	-	1.9	-*	-*
Live, on gills only (?)	1	60	1	0.6	1	1
Total	42	34-67	103+ fragments	27.3	2.5*	1-8

*only intact larvae were included into mean intensity calculations

Most of the live larvae dwelling in the stickleback body cavity were located in the peritoneum (12 larvae), mid-gut (10), and mesentery (9). The remaining nematodes were found on the stomach (8 larvae), pharynx and hind-gut (4 each), liver (2), and oesophagus (1).

The *Anisakis simplex* life cycle involves marine crustaceans, the Euphausiacea. The stage 2 larvae they house are invasive for the second indirect (paratenic?) hosts, i.e., planktivourous fish in which the stage 2 larvae moult into stage 3. The larvae mature sexually in the stomach and intestine of the definitive hosts, seals and whales (SMITH and WOOTTEN 1978). The *A.simplex* life cycle involves also paratenic hosts in which the larvae do not moult. Paratenic hosts serve as a parasite reservoir of a kind, thereby enhancing the nematodes' chances for closing the life cycle. The paratenic hosts are mainly various species of fish and cephalopods (NAGASAWA 1990, ROLBIECKI and ROKICKI 2000).

The *A. simplex* nematodes arrive in the Baltic from the North Sea, predominantly with the herring and, to some extent, with marine mammals. The infected herring migrate to the Baltic to spawn. There are three local herring populations in the Baltic: the spring coastal herring, the spring open sea herring, and the autumn herring. It should be borne in mind that each population contains some individuals from the other two (KOMPOWSKI 1971). The spring coastal herring are most abundant and migrate to the North Sea where they become infected via euphausiids. The population is also the most heavily infected one (GRABDA 1974, MYJAK et al. 1995, ROKICKI et al. 1997).

The stickleback is a small fish (only few individuals exceed 10 cm), common in freshened and brackish areas of the Baltic. As a predator, the species feeds on annelids, crustaceans, insect larvae, molluscs, as well as on the eggs and newly hatched larvae of other fish (WOOTTON 1976). The inshore Baltic waters provide a favourable habitat for the stickleback. Its population here has been observed to steadily increase in abundance since the 1970's (WIKTOR 1976, SKÓRA 1993). For this reason, the stickleback plays an important role in the Baltic ecosystem.

A question arises as to if, and how, the stickleback is involved in the *Anisakis simplex* live cycle in the Baltic.

One of possible infection pathways is for the stickleback its feeding on dead, nematode-infected, fish, e.g., on herring, passing away after strenuous spawning. Another source of infective nematode larvae is in the remains of gutted fish (mainly herring and cod). The Baltic fishermen eviscerate part of their catch on board and throw the viscera overboard. In addition, much of the herring catch is gutted in the harbour. The nematode-infected viscera can be a readily available food for, i.a., the stickleback, as already suggested by PODOLSKA and MOROZIŃSKA (1994).

Crustaceans may be still another pathway of *A. simplex* infestation in the stickleback. It does not seem probable that the stickleback migrates to the North Sea where it could be infected by feeding on the euphausiids (the obligatory intermediate host). However, some species native for the North Sea are sporadically recorded in the Baltic. Perhaps, as contended by GRABDA (1973), the life cycle of some *A. simplex* is confined to the Baltic. That author (GRABDA 1974) found the nematode in the Baltic herring populations which did not migrate to the North Sea where they could have become infected. Most probably, the first intermediate host, the euphausiids, is replaced in the Baltic by other crustacean species. In other seas, *A. simplex* stage 3 larvae had been reported from amphipods and decapods (USPENSKAYA 1963), the paratenic hosts. On the other hand, earlier larvae of the nematode can dwell in other crustaceans, mainly in copepods, and even in cirriped nauplii and metanauplii (SMITH 1983, KOIE 2001). Those are frequently referred to as the so-called transfer hosts, a source of infection for other, larger crustaceans (SMITH 1983). In those, the larvae either persist at their current developmental stage or moult into the next one, the crustacean hosts serving their respective roles as paratenic (transfer) or intermediate hosts. The stickleback could have become infected via both types of crustacean hosts.

The successful experimental infection has provided evidence that transfer of *A. simplex* stage 3 larvae from one host to another is possible. Certainly, a similar transfer takes place under natural conditions as well. The stickleback should then be taken into account as potential *A. simplex* paratenic host. It should also be borne in mind that the fish intended for human consumption could become *A. simplex*-infected by preying on the infected stickleback, thus posing a threat to human health.

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