Larval anisakids parasitizing the blue whiting, *Micromesistius poutassou*, from Motril Bay in the Mediterranean region of southern Spain

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Abstract

A total of 301 blue whiting, *Micromesistius poutassou* Risso, 1826, ranging in length from 17 to 28 cm, from Motril Bay (Mediterranean coast, south Spain) were examined for anisakid nematodes, as these fish are common items in the Spanish Mediterranean diet. Three anisakid species were morphologically identified with a total prevalence of 10.63%. *Anisakis simplex* s.l. Rudolphi, 1809 had a prevalence value of 6.65%, compared with 2.66% for *A. physeteris* Baylis, 1923 and 2.33% for *Hysterothylacium aduncum* Rudolphi, 1802. Variations in prevalence values with season and host size are discussed. Allozyme markers (leucine aminopeptidase-1) were used to identify anisakid nematodes assigned to the *A. simplex* complex and all examined larvae were found to correspond genetically to *A. pegreffii* Nascetti *et al.*, 1986.

Introduction

Anisakids are present in many marine teleosts and the consumption of commercial fish, containing third larval stages (L3) of some anisakid species, constitutes a potential risk for public health. With reference to fish infections, a number of authors have studied the role of marine zooplankton in the transmission of anisakids (Køie, 1993a; Køie *et al.*, 1995; Marcogliese, 1995), although fish may also become infected as a result of predation of other fish infected with L3. Experimental studies on the infection of marine and freshwater fish have shown that the larvae reach the body cavity by penetrating the wall of the stomach or pyloric caecum, infect the general body cavity a few hours later and the musculature after a few days (Smith, 1974). In some fish, such as the herring, the number of larvae of *Anisakis simplex* in the musculature increases after storage (Smith & Wootten, 1975). However, Wootten & Smith (1976) and Smith (1984) showed no migration from the viscera into the flesh of blue whiting once the host is dead. The presence of anisakid larvae in *Micromesistius poutassou* has been previously reported (Wootten & Smith, 1976; Bussmann & Ehrich, 1979; Smith, 1984; Petter & Maillard, 1988; Ruiz-Valero *et al*., 1992; Køie, 1993b). The aim of this survey was to study anisakid infections of the blue whiting, as this fish is frequently consumed in Spain.

Materials and methods

A total of 301 blue whiting (*Micromesistius poutassou*, family Gadidae) from Motril Bay in southern Spain (Mediterranean Sea) were studied from February 1996 to January 1997.

Fish lengths ranged from 17 to 28 cm. Once measurements of total length were made, the fish were dissected, and the free anisakid larvae collected from the body cavity. The viscera, inner organs, and ventral and dorsal musculature were then separately treated with a pepsin-HCl solution (pH 2–2.3) at 37°C, for 2–3 h (modified after McGladdery, 1986). After the larvae were isolated, they were washed with 0.9% NaCl solution and examined under a light microscope. Morphological identification of the anisakids was undertaken using features described by Hartwich (1974), Yoshinaga *et al.* (1987), Petter & Maillard

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(1988), Berland (1989) and Køie (1993b). The L3 of *Anisakis simplex* s.l. were preserved at –70°C until required for subsequent experiments. Larvae were analysed by isoenzyme electrophoresis using thick starch gel (Nascetti *et al*., 1986; Martín-Sánchez *et al*., 1998) in a continuous buffer system (electrode buffer pH 8.6: 661 mM Tris, 83 mM citric acid; gel buffer pH 8.6: 31 mM Tris and 39 mM citric acid). Electrophoretic studies were carried out on single larvae crushed by freeze-thawing in liquid nitrogen and 40 μl of 5% Triton X-100 solution were added. Each gel was stained for leucine aminopeptidase (LAP, EC 3.4.11.1) after Nascetti *et al*. (1986) and the LAP-1 locus was analysed genetically. For statistical analyses of infection parameters, hypothesis tests of proportions based on the normal distribution by calculating the Z_{exp} statistics were used (the differences are significant when $Z_{exp} > 1.96$).

Results

Levels of infection with anisakid larvae

Thirty two fish of the 301 examined were infected with anisakid larvae. Table 1 shows the infection parameters for the three species found. The total prevalence was 10.63%. The highest value was 6.65% for *Anisakis simplex* s.l. (*A. pegreffii* according to electrophoretic studies) which was significantly higher than 2.66% for *A. physeteris* ($Z_{exp} = 2.32$, $P = 0.02$) and 2.33% for *Hysterothylacium aduncum* ($Z_{exp} = 2.56$, $P = 0.01$). The last two species were isolated from eight and seven fish, respectively, and one or two larvae per fish were always isolated. Mixed infections by the three species were only found in one fish, with a prevalence value of 0.33%, whereas combined infections by *A. pegreffii* and *H. aduncum* were recorded in two fish, with a prevalence value of 0.66%. All larvae of the three anisakid species were found in the viscera with the exception of one larva of *A. pegreffii* being found in the ventral musculature.

Seasonal changes in the prevalence, abundance and mean intensity of the three species are recorded in table 2, showing minimal values in the autumn for *A. pegreffii* and in the winter for *H. aduncum* and maximal values in the spring for *A. physeteris*. However, these differences were not statistically significant.

When anisakid infections were related to fish size (table 3), the highest prevalence and abundance by anisakids were found in the largest fish (length $\geqslant 25$ cm). This difference was only significant when comparing the fish groups with shorter and longer lengths ($Z_{exp} = 2.12$, $P = 0.034$). The differences observed between the prevalence for single species were not significant.

Allozyme identification of Anisakis simplex *s.l.*

The 24 larvae morphologically identified as *A. simplex* s.l. were analysed electrophoretically to the diagnostic locus LAP-1. These larvae showed the same alleles (100 and 102) and similar allelic frequencies (0.859 and 0.141, respectively) found in *A. pegreffii* for this locus by Nascetti *et al*. (1986).

Table 1. Prevalence, abundance, and mean intensity of larval anisakids in the blue whiting.

Parasite	NIH	n	P	A	I(R)
Anisakids	32	42	10.63	0.14	1.31(1–4)
Anisakis pegreffii	20	24	6.65	0.08	1.20(1–4)
A. physeteris	8	10	2.66	0.03	1.25(1–2)
Hysterothylacium aduncum	7	8	2.33	0.03	1.14(1–2)

NIH, number of infected hosts; n, number of larval anisakids; P, prevalence; A, abundance; I, mean intensity; R, range.

Table 2. Seasonal variation in the prevalence, abundance, and mean intensity in larval anisakids in the blue whiting.

Parasite	Parameters	Winter	Spring	Summer	Autumn
Anisakids	P	9.47	14.04	13.70	6.58
	A	0.13	0.21	0.16	0.08
	I	1.33	1.50	1.20	1.20
Anisakis pegreffii	P	6.32	8.77	9.59	2.63
	A	0.09	0.09	0.11	0.03
	I	1.50	1.00	1.14	1.00
A. physeteris	P	2.11	5.26	2.74	1.32
	A	0.02	0.09	0.03	0.01
	I	1.00	1.67	1.00	1.00
Hysterothylacium aduncum	P	1.05	3.51	2.74	2.63
	A	0.01	0.04	0.03	0.04
	I	1.00	1.00	1.00	1.50

P, prevalence; A, abundance; I, mean intensity.

Table 3. Variations in the prevalence, abundance, and mean intensity in larval anisakid infection of blue whiting with host length.

Fish length (N)	Parasite	Prevalence	Abundance	Mean intensity
17–18 cm	Anisakids	4.16	0.04	1.00
(48)	*Anisakis pegreffii*	2.08	0.02	1.00
	A. physeteris	2.08	0.02	1.00
	Hysterothylacium aduncum	–	–	–
19–20 cm	Anisakids	11.53	0.13	1.16
(52)	*A. pegreffii*	5.76	0.06	1.00
	A. physeteris	1.92	0.01	1.00
	H. aduncum	3.84	0.05	1.50
21–22 cm	Anisakids	7.81	0.12	1.60
(64)	*A. pegreffii*	6.25	0.10	1.60
	A. physeteris	1.56	0.01	1.00
	H. aduncum	–	–	–
23–24 cm	Anisakids	10.00	0.10	1.00
(60)	*A. pegreffii*	6.66	0.06	1.00
	A. physeteris	–	–	–
	H. aduncum	3.33	0.03	1.00
⩾ 25 cm	Anisakids	16.88	0.24	1.46
(77)	*A. pegreffii*	10.38	0.11	1.12
	A. physeteris	6.49	0.07	1.20
	H. aduncum	3.89	0.03	1.00

N, number of examined hosts.

Discussion

Three anisakid species, *Anisakis pegreffii*, *A. physeteris* and *Hysterothylacium aduncum*, were identified in the blue whiting collected from Motril Bay in western Mediterranean Sea, off southern Spain.

According to Nascetti *et al*. (1986) and Mattiucci *et al*. (1997), *A. simplex* is a complex of three sibling species, which are reproductively isolated. Of these, *A. simplex* s.s. is mainly found in the Atlantic Ocean whereas *A. pegreffii* principally inhabits the Mediterranean Sea although specimens of the two forms can coexist in the same area and even in the same host. In the present study, 42 anisakid larvae isolated from the blue whiting were in the third larval stage, 24 of which were morphologically identified as *A. simplex* s.l., and electrophoretically identified as *A. pegreffii*.

The prevalence of anisakids in the blue whiting from Motril Bay is much lower than those (63–100%) occurring in specimens examined from the Atlantic Ocean (Wootten & Smith, 1976; Sanmartín *et al.*, 1989; Ruiz-Valero *et al*., 1992). Moreover, Ruiz-Valero *et al*. (1992) reported a prevalence of 23.5% for *A. simplex* s.l. in fish collected from the Spanish Mediterranean coast, while in the present study, this prevalence value was even lower (6.65% for *A. pegreffii*).

In this survey, the prevalence of infection by *H. aduncum* was 2.33%, whereas in a previous report for fish collected along the Spanish Mediterranean coast no infection by *H. aduncum* was recorded (Ruiz-Valero *et al*., 1992). However, the prevalence in blue whiting from the Spanish Atlantic coast was 22.4% (Ruiz-Valero *et al*., 1992) and 23.9% (Sanmartín *et al*., 1989).

Ruiz-Valero *et al*. (1992) also reported an increase in the prevalence of *A. simplex* s.l. and *H. aduncum* in blue whiting marketed in Granada during spring and summer. However, in the present study, no significant differences in infections could be attributed to seasonal variation (table 2), presumably due to the low levels of anisakid infection. On the other hand, a higher prevalence and abundance in hosts longer than 25 cm were observed (table 3). An increase in the prevalence of anisakids in larger blue whiting and other species has been recorded (Bussmann & Ehrich, 1979; McGladdery & Burt, 1985; Ruiz-Valero *et al*., 1992; Adroher *et al*., 1996), and this could be due to an accumulation of parasites in the host throughout its life (Bussmann & Ehrich, 1979).

In recent years, several cases of anisakidosis have been reported in Spain and one, at least, in a patient who frequently consumed hake and blue whiting (Clavel *et al*., 1993). Also, Yagi *et al.* (1996) described a woman patient with diarrhoea and abdominal pain, which was followed by the expulsion of one adult worm of *H. aduncum*. However, only one *A. pegreffii* larva (< 5% larvae of all three species) occurred in the fish musculature.

Finally, the low prevalence of anisakids in fish studied in this survey (10.63%), especially in the muscle (0.33%), and the low mean intensity of infection (1.31) clearly indicate that the risk of contracting anisakidosis from consumption of blue whiting captured from Motril Bay in the southern Spain (western Mediterranean Sea) is very small.

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