

EXPERIMENTAL STUDIES ON THE MIGRATION OF *ANISAKIS* SP. LARVAE (NEMATODA : ASCARIDIDA) INTO THE FLESH OF HERRING, *CLUPEA HARENGUS* L.

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Abstract—SMITH J. W. & WOOTTEN R. 1975. Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridida) into the flesh of herring, *Clupea harengus* L. *International Journal for Parasitology* 5: 133–136. In two experiments, a batch of 38 herring from Shetland was gutted immediately on capture (0 h) and further batches were gutted 14 and 37 h after capture and storage on ice. The numbers of *Anisakis* larvae in the body-cavity and in the flesh (detected by pepsin-HCl digest) were then counted. The proportion of fish with worms in the flesh was higher at 14 and 37 h than at 0 h. The percentage of the total worm burden in the flesh increased between each time interval, which suggests that a large-scale migration of larvae into the flesh occurs in ungutted fish. There was a positive association between the numbers of larvae in the viscera and the numbers in the flesh. Most larvae occurred in the hypaxial muscles. The results are discussed in relation to the observations of earlier workers.

INDEX KEY WORDS: *Anisakis* sp. larvae; larval migration; herring flesh; pepsin-HCl digest; North Sea.

INTRODUCTION

THE PRESENCE of *Anisakis* sp. larvae in food fish achieved public health significance some 14 y ago when consumption of live larvae with raw or inadequately cooked fish was first reported to cause eosinophilic granuloma in the alimentary tract of man (see, for example, Oshima, 1972). The numbers of larvae in the flesh of fish is of importance from the medical viewpoint.

There is controversy in the literature concerning the occurrence of *Anisakis* larvae in the flesh of fish at capture and also regarding the ability of larvae to migrate from the body-cavity into the flesh after capture and death of the host (Van Thiel, Kuipers & Roskam, 1960; Roskam, 1960; Vik, 1966; Khalil, 1969; Davey, 1972). It was decided, therefore, to investigate the numbers of larvae in the flesh of herring, *Clupea harengus* L., at capture and at intervals after storage of ungutted fish on ice.

MATERIALS AND METHODS

The herring used in the two experiments described in this paper were caught by commercial purse-seine vessels off Balta Sound, East Shetland, in July 1971. In both experiments a batch of 38 herring was gutted immediately on capture and further batches gutted 14 and 37 h after capture. The gutted and ungutted fish were stored in flake ice until gutting and/or digestion. The temperature of herring in the ice rose to 3°C during the first 14 h, and reached 10°C during the next 23 h. The temperature rise between 14 and 37 h is higher than that recommended for commercial practice.

The digest technique used was a modification of that developed by Stern, Chakravarti, Uzmann & Hesselholt (1958) for use with salmon. In our experiments a digest solution was made up with 10 g of 1:2500 pepsin powder (BDH) and a litre of 1% HCl. The length and gutted weight of each herring was taken and the head removed. The fish were from 21 to 32 cm long and gutted weights ranged from 60 to 250 g. Each fish was then washed to remove any nematodes lying loose in the body-cavity or adhering to the external surfaces. It is estimated that up to 10 per cent of the visceral burden of worms may be lost during gutting and washing. This estimate was arrived at by washing fish gutted after 60 h into sieves and counting worms washed off. The walls of the body-cavity were then inspected for worms partially embedded in the flesh; such worms were counted and left in place. Each herring was then split, the vertebral column removed, and the fillets placed for 40 min in a 2-litre beaker containing the digest solution in a water-bath at 52°C. The solution was stirred occasionally to break up the fillets. The beakers were then removed from the water-bath and their contents poured through a commercially available nylon sieve (2 mm mesh, knot to knot). The sieve was irrigated with a jet of water to break up and wash through muscle tissue. After sieving, any worms present were counted against a dark background and their identity checked using a stereomicroscope. There is no evidence that any worms are lost during the digestion and sieving processes. All partially embedded worms known to have been introduced into the digest solution were recovered during sieving.

The viscera of the herring were preserved in 4% formal-saline until examination; the viscera were dissected and the worms counted using a stereomicroscope.

The hypaxial and epaxial muscles of a sample of 50 herring from the Shetlands were digested separately in

order to determine the number of *Anisakis* larvae in each part of the flesh.

A further four samples of herring caught commercially in the Shetland, North Minch, South Minch and Clyde areas were processed as described above in order to compare the numbers of worms in the flesh and viscera.

RESULTS

All larvae recovered in the experiments morphologically resembled the third-stage larvae designated *Anisakis* sp. larva (1) by Berland (1961). Such larvae were reared *in vitro* to the adult stage by Van Banning (1971) and identified as *Anisakis simplex* (of which *A. marina* is a synonym according to Davey, 1971).

The proportions of herring with and without *Anisakis* larvae in the flesh at each time interval for both experiments are shown in Table 1. The pro-

TABLE 1—PROPORTIONS OF 38 HERRING WITH *Anisakis* LARVAE IN THE FLESH (%)

		0 h	14 h	37 h
Expt 1	Flesh infected	44.7	60.5	73.7
	Flesh uninfected	55.3	39.5	26.3
Expt 2	Flesh infected	39.5	68.4	68.4
	Flesh uninfected	60.5	31.6	31.6

portions infected in the three periods are significantly different ($P < 0.05$) in both experiments, with a higher proportion of fish gutted after 14 and 37 h being infected than at 0 h. Table 2 shows the num-

TABLE 2—TOTAL NUMBERS OF *Anisakis* LARVAE IN HERRING (T); NUMBER IN VISCERA (V) AND NUMBER IN FLESH (F); TOTAL WORM BURDEN IN FLESH (% F)

		0 h	14 h	37 h
Expt 1	T	848	666	521
	V:F	814:34	621:45	461:60
	% F	4.01	6.76	11.52
Expt 2	T	513	438	336
	V:F	492:21	384:54	269:67
	% F	4.09	12.33	19.94

bers of worms in both viscera and flesh at each time interval and the percentage of the total worm burden occurring in the flesh. The percentage of the total worm burden in the flesh increased between each time interval in both experiments.

The frequency distributions of the numbers of *Anisakis* larvae in the flesh of herring at each time interval for both experiments are given in Table 3.

TABLE 3—FREQUENCY DISTRIBUTION OF *Anisakis* LARVAE IN HERRING FLESH, AND MEAN INTENSITY OF INFECTION

	No. of worms	Frequency		
		0 h	14 h	37 h
Expt 1	0	21	15	10
	1	7	10	9
	2	7	8	11
	3	1	3	3
	4	1	0	5
	5	0	2	0
	≥ 6	1	0	0
	Totals	38	38	38
	Mean intensity	0.895	1.184	1.579
Expt 2	0	23	12	12
	1	11	10	10
	2	3	9	6
	3	0	4	4
	4	1	2	1
	5	0	0	2
	≥ 6	0	1	3
	Totals	38	38	38
	Mean intensity	0.553	1.421	1.763

In all cases the observed distribution did not differ significantly from a Poisson distribution. The mean intensities of infection were then compared in a two-way analysis of variance. Individual counts were transformed using the transformation $y = \sqrt{(x + \frac{1}{2})}$. Mean intensities of infection from the analysis of variance are shown in Table 4. In

TABLE 4—MEAN INTENSITY OF INFECTION WITH *Anisakis* LARVAE AFTER TRANSFORMATION [$y = \sqrt{(x + \frac{1}{2})}$]

	0 h	14 h	37 h
Expt 1	0.901	1.189	1.588
Expt 2	0.686	1.430	1.643

both experiments the mean intensity of infection at 0 h was significantly lower than that of the average of 14 and 37 h ($P < 0.05$ for experiment 1, and $P < 0.001$ for experiment 2). A direct comparison of the mean intensities at 14 and 37 h showed no significant difference.

With the data collected it was not possible to determine the relationship between level of flesh infection and time; there might have been a continuous increase in mean intensity throughout the time period considered, or the level of infection might have stabilized after 14 h. In experiment 2 there was an appreciable difference in the rate of increase in the mean intensity of infection over the two time periods, the rate of increase between 0 and 14 h being greater than that between 14 and 37 h. In experiment 1, however, there was little difference in the rate of increase between the two periods.

Association between the numbers of *Anisakis*

larvae in the viscera and in the flesh of herring at each time interval for both experiments was tested using Kendall's rank correlation coefficient (τ). In both experiments for all time intervals the rank correlation coefficients were statistically significant, showing a positive association between the numbers of *Anisakis* larvae in the viscera and in the flesh. This group of correlation coefficients may be regarded as homogeneous and a mean of 0.39 applies to all sets.

Much larger numbers of *Anisakis* larvae were found in the hypaxial muscles than in the epaxial muscles of a sample of 50 herring in which the two sets of muscles were digested separately. Of the 48 larvae found in the flesh of these fish, 43 (89.6 per cent) were located in the hypaxial muscles and only 5 (10.4 per cent) occurred in the epaxial muscles.

The percentage of the total worm burdens found in the flesh of fish examined after 37 h in both experiments and the percentage in the flesh of other herring samples from Shetland, North Minch, South Minch and Clyde, gutted after a similar time interval, are shown in Table 5. Although the per-

TABLE 5—TOTAL WORM BURDENS AND PERCENTAGE OF WORM BURDEN IN FLESH OF HERRING SAMPLES GUTTED 36–48 h AFTER CAPTURE IN DIFFERENT AREAS

Sample	Total worm burden	% in flesh	Time of gutting after capture (h)
Expt 1	521	11.5	37
Expt 2	337	19.9	37
Shetland	673	9.7	38
North Minch	264	8.0	36
South Minch	283	13.4	36
Clyde	15	20.0	48

centage varied between samples, in all cases an appreciable proportion of the total worm burden was present in the flesh.

DISCUSSION

An appreciable number of *Anisakis* larvae was present at the time of capture in the flesh of the herring used in our experiments. However, Van Thiel *et al.* (1960) and Vik (1966) did not find larvae in the flesh of North Sea herring and mackerel, respectively, which were gutted immediately after capture. Furthermore, Khalil (1969) found only 2 per cent of the total worm burden partially embedded in the flesh of North Sea herring at the time of capture.

A large-scale migration of *Anisakis* larvae from the viscera to the flesh of the herring occurred in both our experiments so that, in the second experiment, almost 20 per cent of the total worm burden was present in the flesh after 37 h. This situation contrasts with that recorded by Khalil (1969) who

found no increase in the number of larvae partially embedded in the flesh of herring left ungutted for 3 days after capture. Moreover, Van Thiel *et al.* (1960) found only a very small increase in the number of partially embedded larvae in herring kept for 4–8 days before gutting. Khalil (1969) did find that storage of herring before gutting increased the number of free larvae in the body-cavity. Davey (1972) reported less than 1 per cent of the total number of *Anisakis* larvae present in herring from the North Sea and west of Scotland to be penetrating the flesh despite the time that must have elapsed between capture and examination. A migration of *Anisakis* larvae into the flesh of the host comparable to that described in the present study was observed in North Sea mackerel by Vik (1966). Although no larvae were present in the flesh of mackerel when caught, Vik found that after 3–4 days 10–13.6 per cent of the total number of larvae had migrated to the flesh.

The inconsistencies between the results of the present study and those of most previous workers may be due in part to the different temperatures at which fish were kept. However, it is likely that the discrepancies are due mainly to differences in the techniques used to detect larvae in the flesh. Most workers have examined the flesh of fish with the naked eye, supplemented in some cases by slicing and candling. The flesh of herring is dark and oily and the small larvae of *Anisakis* are extremely difficult to detect by such methods and often only partially penetrated larvae are observed. During the present study such partially penetrated larvae represented only 3.8 per cent of the total number found in the flesh. Stern *et al.* (1958) found that a pepsin-HCl digest technique recovered 21.9 per cent more *Anisakis* larvae from the flesh of salmon than manual methods. There is no doubt that digest techniques are the only efficient means of recovering nematode larvae from the dark flesh of fish like herring. It is significant that Roskam (1960) used a digest method and reported that the percentage of the total worm burdens found in the flesh per 100 North Sea herring ranged from 2.5 to 14.4 per cent in different size groups of fish; these figures are comparable with those obtained during the present study.

The factors causing excapsulation and migration of *Anisakis* larvae after death of the host fish are unknown, but presumably relate to physico-chemical changes in the viscera. A rise in temperature might be expected to cause increased activity since the third-stage larvae of *Anisakis* complete their life-cycle in a warm-blooded mammal. However, the rise in temperature of fish kept on ice was not very great, particularly during the first 14 h, and other factors may be responsible.

The presence of most *Anisakis* larvae in the hypaxial muscles of the herring may be due to the fact that these muscles surround the body-cavity and are therefore the first part of the musculature

to be contacted by migrating larvae. The larvae apparently do not migrate in large numbers into the epaxial muscles. Young (1972) found that most *Anisakis* larvae in the flesh of cod occurred in the hypaxial muscles and a similar distribution occurs in whiting (Wootten, unpublished observations). Novotny & Uzzmann (1960) reported that 47 per cent of chum salmon had no *Anisakis* larvae in the epaxial muscles; 90 per cent of those that had larvae in the epaxial muscles showed less than 10 per cent of the total infection in that site.

A significant proportion of the total numbers of *Anisakis* larvae occurred in the flesh of herring in all Scottish waters. The positive association found between the numbers of larvae in the viscera of herring and the numbers in the flesh will result in there being larger numbers in the flesh of fish in areas of heavy *Anisakis* infection, like Shetland, than in areas of medium and low infection, like the Minches and Clyde.

Herring caught by Scottish vessels are not gutted at sea and a period of at least several hours elapses before fish are landed and the viscera removed. There will be, therefore, some migration of *Anisakis* larvae into the flesh to add to those already present at the time of capture. In order to eliminate any health risk to the consumer of raw or lightly-salted herring it is obviously desirable to enforce measures to kill larvae within the flesh, e.g. deep freezing to -20°C for 24 h (Ruitenbergh, 1970).

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