Journal of Food Protection, Vol. 56, No. 9, Pages 783-787 (September 1993)

Catch Handling and the Possible Migration of *Anisakis* Larvae in Herring, *Clupea harengus*

ALLAN ROEPSTORFF[1]*, HORST KARL[2], BOUKE BLOEMSMA[3], and HANS H. HUSS[1]*

[1]*Technological Laboratory, Ministry of Fisheries, Technical University, blgd. 221, DK-2800 Lyngby, Denmark;*
[2]*Bundesforschungsanstalt für Fischerei, Institut für Biochemie und Technologie, Palmaille 9, 2000 Hamburg 50, Germany; and*
[3]*TNO CIVO-Institutes, P.O.Box 183, 1970 AD IJmuiden, The Netherlands.*

(Received for publication July 27, 1992)

ABSTRACT

The behavior of larvae of *Anisakis* sp. in North Sea herring (Clupea harengus) after capture was examined by a sensitive quantitative method (digestion). In one winter and one summer experiment, the mean numbers of worms in freshly caught herring were 0.06 and 0.09 (in double fillets), 0.19 and 0.24 (in double belly flaps), and 10.4 and 7.8 larvae (in viscera), respectively. In each experiment herrings were stored ungutted for up to 5 1/2 d on ice (0°C), in refrigerated/chilled sea water (-1-0°C), or in warm sea water (10°C), but no changes in the numbers of *Anisakis* larvae in the belly flaps and the fillets could be demonstrated.

The present results show that *Anisakis* larvae are present in the flesh of herring already at capture, but no significant postmortem migration into the flesh could be demonstrated during storage. Thus, immediately gutting on board cannot eliminate or even reduce the risk from eating raw or inadequately processed herring.

The presence of third-stage larvae (L3) of the herring worm, *Anisakis* sp., in the flesh of marine teleost fish has considerable practical impact, as the larvae constitute a potential public health problem as well as an aesthetic problem. Since the first reports on the zoonotic disease Anisakiasis appeared in 1960 (*14,18*), several human cases, caused by consuming raw or inadequately prepared marine fish containing infective larvae, have been described from most parts of the world.

After an infected first intermediate host is ingested by the live fish, the larvae penetrate the intestinal wall. The larvae may then encoil on the surface of the internal organs, or they may migrate into the flesh. A similar migration from the viscera into the flesh has been described after the fish have been caught (i.e., postmortem) for the fatty species mackerel (Scomber scombrus) (*15,19*) and herring (Clupea harengus) (*16*), while not for lean fish species, such as whiting (Merlangius merlangus) (*15*), blue whiting (Micromesistius poutassou) (*15*), and walleye pollock (Theragra chalcogramma) (*3*). However, some controversy exists, as other studies (*7,8*) have not been able to demonstrate any postmortem migration in herring.

Recently, public interest has focused on the Anisakis problem, and suggestions have been that increased numbers of parasites were found in the flesh due to modern handling practices such as rapid cooling and storage of ungutted fish (herring, mackerel) in chilled sea water. Therefore, it has also been suggested that these fish should be gutted on board immediately after capture to reduce the number of nematodes in the fillets.

The present European Economic Community (EEC) collaborative work was carried out with the objective to study the migration behavior of *Anisakis* larvae after capture of the herring. The effect of rapid chilling and delays in the application of chilling as well as the chilling method was studied.

MATERIALS AND METHODS

Some details of the fish material are presented in Table 1, while the experimental design is summarized in Table 2.

The two experiments were carried out on lean winter herring (Exp. 1) and fat summer herring (Exp. 2), respectively. The herrings were caught by a commercial purse seine vessel and were immediately placed at different storage conditions. Gutting and filleting of the control groups were carried out 0-3 h after capture, while the gutting and filleting of the stored groups were carried out 1 1/2 and 5 1/2 d later.

The storage conditions were: 'Ice': The herrings were placed in drained polystyrene boxes completely surrounded by ice. 'Refrigerated sea water/chilled sea water (RSW/CSW)': The herrings were stored in refrigerated sea water in tanks on board the fishing vessel. The temperature of the tank water was -1°C. On landing after 24-26 h, the fish were transferred to chilled sea water in insulated containers (0°C). 'Warm sea water': The herrings were

TABLE 1. *Sampling data.*

Experiment	Date of capture	Location	Position	Length[a] cm	%Fat[b] content
1	March 2, 1990	East of Shetland	61,04°N 01,10°E	24.5±1.1 (21.5-28.0)	2.5±2.8 (<0.5-10.0)
2	August 4, 1990	Western North Sea	55,36°N 01,04°E	24.5±1.3 (21.0-29.5)	14.6±3.8 (7.0-20.5)

[a] All fish: mean ± SD (minimum-maximum).
[b] Subsample (20 fish): mean ± SD (minimum-maximum).

TABLE 2. *Summary of experimental design.*

Experiment	Storage condition	Temperature (°C)	Storage time (h/d)[b]	No. of fish examined[b]
1	At capture	-	0-3 h	124
	Ice	0	1 1/2 + 5 1/2 d	50+50
	RSW/CSW	≤0	1 1/2 + 5 1/2 d	50+50
	Warm sea water	10 ± 1	1 1/2 + 5 1/2 d	50+50
	Delayed ice[a]	10/0[a]	5 1/2 d	50
2	At capture	-	0-3 h	75
	Ice	0	1 1/2 + 5 1/2 d	50+50
	RSW/CSW	≤0	1 1/2 + 5 1/2 d	50+50
	Warm sea water	10 ± 1	1 1/2 + 5 1/2 d	50+45
	Delayed ice[a]	10/0[a]	5 1/2 d	40

[a] Delayed ice: 12 h in warm sea water (10°C) followed by 5 d in ice (0°C).

[b] Two values represent 2 different times of filleting.

stored in insulated containers with sea water, of which the temperature was regulated to 10 ± 1°C by adding ice or hot water. 'Delayed ice': The herrings were kept for 12 h in warm sea water (10 ± 1°C) followed by 5 d on ice.

In each experiment a small lot of herrings was placed in a freezer (-20°C) immediately after capture for later estimation of fat content. After the storage periods indicated in Table 2, all herrings were filleted by hand and divided into three separate fractions: Viscera (the viscera and loose worms washed out from the body cavity), the belly flaps (the flesh surrounding the body cavity-this fraction is discarded commercially), and the fillets. These fractions were frozen (-20°C) individually for later laboratory examinations.

In the laboratory all fractions were digested individually in pepsin-HCl (50 ml concentrated HCl, 12.5 g pepsin (Orthana, 10.000 NF), 45 g NaCl, and tap water to 5 L). After disintegration for 1 min in a stomacher, the samples were digested for 2-4 h at 42°C and poured through a 500-μ sieve, followed by counting of the worms against a black background.

In both experiments large numbers of larvae were all identified as *Anisakis* sp. by microscopical examination of morphology (according to the criteria in ref. *17*), and therefore, all larvae were considered to belong to this genus. Abundance is calculated as mean worm burden of all fish examined, including noninfected specimens (according to the definition in ref. *9*).

STATISTICS

All data were analyzed by the Wilcoxon Rank test (the NPAR1WAY procedure of the Statistical Analysis System [SAS]) (*2*) to test that the different groups of fish within each experiment do not differ from each other with respect to the fish population and the total worm burden (only significant differences are mentioned in the text). Thereafter, the probability of the individual nematodes for being located in the fillet, the belly flaps, or the viscera was tested by use of a logistic regression model (the CATMOD procedure in SAS) (*2*). As the response variable in the catmod procedure is a 0-1 variable, the models tested were both location in 'fillet' versus 'belly flaps + viscera' and in 'fillet + belly flaps' versus 'viscera', thus giving rise to two models per condition of storage of each experiment.

The variables tested in the individual models were 'Time of storage', 'length of the herrings', and 'total worm burden'. In the catmod procedure the effect of each variable was tested by a likelihood ratio test using a chi-square approximation to the deviance. When this test indicated an overdispersion (i.e., chi-square > DF), the chi-square values were corrected with the overdispersion factor (the chi-square:DF ratio) (*10*). The catmod analyses were carried out by backward selection, i.e., stepwise elimination of the most nonsignificant variables, ending up with models containing only significant ($p < 0.05$) variables.

RESULTS

In Table 3, the abundance and the relative distributions of nematode larvae are shown for the two experiments.

Experiment 1

The large majority of lean winter herrings (92.0%) were infected with *Anisakis* larvae. Table 3 shows clearly that there were no differences in the location of larvae between the control group gutted immediately after capture and the groups of fish gutted after various storage conditions, indicating that no systematic migration occurred during storage. This was confirmed statistically, as 'storage time' was not significant in any of the models of logistic regression, irrespectively, if the location in 'fillet' or in 'fillet + belly flaps' was considered. As no significant differences between groups could be observed, the data from all fish could be compiled, and thus, the overall abundance was 8.4 nematode larvae, of which 0.04 (0.5%) were found in the fillets, and 0.19 (2.3%) were found in the belly flaps. The individual worm burdens varied from 0 to 75 and showed a clear overdispersion of nematodes within the host population.

The total worm burden increased with the length of the fish host, but at the same time the relative number of larvae in the flesh decreased (Fig. 1). This corresponds very well with the statistical analyses, which showed that the individual nematode larvae were less prone to be situated in 'fillets + belly flaps', when the total worm burden of the host was large, while the individual larva was more often situated in 'fillets and belly flaps', when the total worm burden was small ($p < 0.05$ in all 4 models, i.e., at the four storage conditions). The location of the larvae was in no case significantly correlated with the length of the herrings. It may be calculated from the mean of estimates from the four models that the odds ratios (OR, which are approximations to the relative risks) for being located in 'fillets + belly flaps' for some selected total worm burdens were: 1 worm: OR = 1.26, 10 worms: OR = 1, 20 worms: OR = 0.77, 30 worms: OR = 0.60, and 40 worms: OR = 0.46. The 'total worm burden' was only significantly correlated (negatively, $p < 0.05$) with the probability for a location in the 'fillet' (versus 'viscera + belly flaps') at one of the storage conditions (10°C).

Experiment 2

The fat summer herrings had largely the same size and distribution of the parasite population as the winter herrings. The results, presented in Table 3, indicated that no migration of larvae into the flesh occurred during storage, irrespective of storage condition and storage duration. One group (stored at 10°C for 5 1/2 d) had significantly lower total worm burden in the viscera compared to the 'at capture' group (Wilcoxon Rank test, $p < 0.001$), while the number of larvae in the flesh was similar to the other

TABLE 3. *The abundance (Abun.) and the relative distribution (%) of* Anisakis *larvae in the different fractions of herrings from Exp. 1 (winter herring) and Exp. 2 (summer herring).*

Experiment	Storage conditions	Fillets		Belly flaps		Viscera		Total	
		Abun.	%	Abun.	%	Abun.	%	Abun.	min-max
Exp. 1	At capture	0.06	0.6	0.19	1.8	10.35	97.6	10.60	0-71
	Ice (1 1/2 d)	0.02	0.3	0.12	1.7	6.90	98.0	7.04	0-42
	Ice (5 1/2 d)	0.00	0.0	0.14	2.1	6.56	97.9	6.70	0-47
	RSW/CSW (1 1/2 d)	0.04	0.6	0.20	2.8	6.96	96.7	7.20	0-55
	RSW/CSW (5 1/2 d)	0.06	0.7	0.28	3.4	7.80	95.8	8.14	0-75
	10°C (1 1/2 d)	0.08	0.9	0.18	1.9	9.00	97.2	9.26	0-59
	10°C (5 1/2 d)	0.02	0.3	0.16	2.2	7.00	97.5	7.18	0-32
	Delayed ice (5 1/2 d)	0.02	0.2	0.28	3.4	7.98	96.4	8.28	0-30
Exp 2	At capture	0.09	1.1	0.24	2.9	7.83	95.9	8.16	0-60
	Ice (1 1/2 d)	0.06	1.2	0.12	2.4	4.92	96.5	5.10	0-20
	Ice (5 1/2 d)	0.06	1.2	0.32	6.2	4.76	92.6	5.14	0-37
	RSW/CSW (1 1/2 d)	0.04	0.5	0.24	2.9	8.00	96.6	8.28	0-63
	RSW/CSW (5 1/2 d)	0.00	0.0	0.12	2.0	5.84	98.0	5.96	0-29
	10°C (1 1/2 d)	0.08	0.8	0.14	1.4	10.10	97.9	10.32	0-56
	10°C (5 1/2 d)	0.02	0.8	0.33	12.4[a]	2.33[a]	86.8	2.69[a]	0-15[a]
	Delayed ice (5 1/2 d)	0.03	0.4	0.10	1.7	5.63	97.8	5.75	0-44

[a] Number of larvae in viscera underestimated, see text.

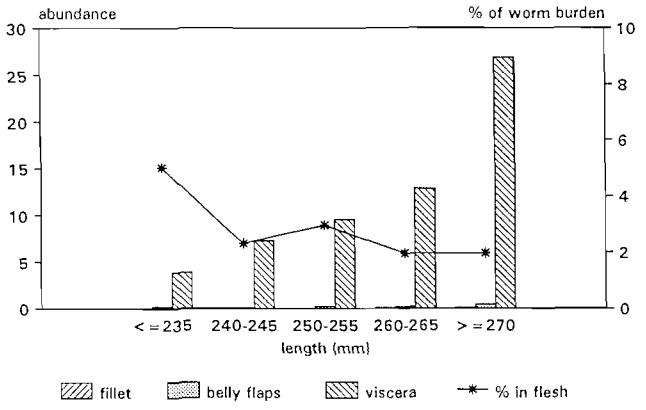

Figure 1. *Distribution of* Anisakis *larvae in lean winter herring (Exp. 1) in relation to the length of the fish. Columns and left axis: Abundance (mean worm burden) in the different fractions. Curve and right axis: The relative number of larvae in the flesh (fillets + belly flaps).*

groups. This may be explained by the fact that when filleting these fish, it was observed that the viscera was largely disintegrated, and many of the larvae were loose within the body cavity. Furthermore, several larvae were found on the outer surface of these herrings (e.g., on the gill lids), indicating that a large part may have escaped from the hosts through the anal and/or the gill region (few fish with a rupture through the abdominal wall were discarded).

The analysis of logistic regression (the '10°C for 5 1/2 d' group was discarded from these analyses, see above) showed that no variables could significantly explain the position in 'fillets', while the 'total worm burden' was significant in three of the models concerning the position in 'fillets + belly flaps' ($p < 0.05$, nearly the same correlation estimates as in Exp. 1). In one model ('delayed ice'), the 'length of the host' was significant instead of 'total worm burden', illustrating that these two variables are competitive with respect to the probability of the larvae to be located in the flesh. The most interesting variable, 'storage time', was significant ($p < 0.01$) in one analysis (10°C for 1 1/2 d), but here the percentage of larvae in the flesh was lower after storage than in the 'at capture' group (i.e., no migration into the flesh). The reason may most probably be the rather high number of larvae in the viscera of the day 1 1/2 group.

DISCUSSION

The main purpose of the present study was to get more comprehensive experience about the possible postmortem migration of *Anisakis* larvae from the viscera into the flesh of herring and about the factors influencing such migration. Previous studies have shown rather conflicting results with respect to the occurrence of this phenomenon, but nevertheless, its existence has been generally accepted. Thus, officially recommended prevention measures of anisakiasis include 'evisceration fish as soon as possible after catch to stop additional larvae from moving into the edible musculature' (*1*), and there exists a risky belief in the 'promptly fillet and keep the doctor away' strategy (*12*).

The best studied fish species with respect to postmortem migration is the herring, in which an early study (*18*) by dissection demonstrated weak evidence for migration. More convincing experiments, carried out by digestion of the flesh, showed that a significant migration from viscera into flesh (belly flaps + fillets) took place in herring left on ice for 14 and 37 h (final temperature 10°C), as the mean number of larvae in the flesh increased from 0.9 to 1.6 in one experiment and from 0.6 to 1.8 in the replicate experiment (*16*). It has also been shown that *Anisakis* larvae may migrate into the flesh during cold smoking, carried out without previous removal of the visceral larvae (*6*). In contrast, other studies have not been able to show any signs

of a postmortem migration in herrings, as no increase in the number of larvae in the flesh was found after 1-3 d on ice (final temperature: 5-10°C) (*8*), and as only constant low numbers were found in the flesh of commercially landed herrings (i.e., at least 1 to a few d after catch) (*5*). These discrepancies have been explained (*16*) by suboptimal methods (nondigestion) used in the latter experiments, which failed to show migration, but recently it has been shown (*7*) that even the application of a digestion technique may not always result in a demonstration of postmortem migration.

With respect to other fish species, a migration into the flesh after capture has been shown in the mackerel (*15,19*), and in the latter work the mean number of larvae in the flesh increased significantly from 2.4 to 5.0 larvae after 24 h on ice. In contrast, a postmortem increase in the number of larvae in the flesh has not been demonstrated in other fish species, such as whiting (*15*), blue whiting (*15*), walleye pollock (*3*), and chilean hake, Merluccius gayi (*4*). This inconsistency in the occurrence of migration in stored round fish led to the hypothesis (*15*) that the *Anisakis* larvae were only stimulated to migrate in 'fatty' fish species (i.e., herring and mackerel, in which the main depot lipids are found in the flesh), while comparable stimuli were not found in 'nonfatty' species (i.e., the gadiform fish, in which lipids are stored mainly in the liver or mesenteries).

The present experiments were not able to demonstrate any tendency of postmortem migration into the flesh of herrings, irrespective of storage condition and storage duration (Exp. 1 and 2). Thus, not even storage at 10°C, at which the larvae presumably have optimal conditions for migration, gave any increase in the number of larvae in the flesh after 1 1/2 and 5 1/2 d, despite the viscera during that period becoming totally disintegrated, and that many larvae after 5 1/2 d had migrated out of the fish through the anal or the gill openings in the summer experiment. Therefore, the present experiments together with the older studies on herring indicate that although postmortem migration may occur in certain circumstances, this migration may not be the rule. It is difficult to explain the different findings of the various studies, but the present study seems to indicate that season and physiological constitution of the fish may only have minor influence on the postmortem behavior of the larvae.

Also, the distribution of *Anisakis* larvae within the body of fresh caught fish seems to vary with the species and local population of the hosts and to the method used for the quantification. Thus, *Anisakis* larvae in some early studies, using nondigestion methods, were not found at all in the flesh of live herring (*18*) or live mackerel (*19*). Using the more reliable digestion methods, it has now been common to find 1-4% of the total worm burdens in the flesh of herring (*6,7,16*), but the relative frequency may vary considerably from stock to stock (geographical area, parasitic source) (*11*). In other fish species the relative number of larvae situated in the flesh at capture may be of similar magnitude (mackerel [*15*], chilean hake [*4*] and walleye pollock [*3*]), or the relative occurrence in flesh may be somewhat larger (10-20% in cod, Gadus morhua [*20*] and blue whiting [*15*]). In whiting, as much as 60-70% of the larvae are found in the flesh immediately after capture, which in actual numbers were 72 larvae in the flesh of each fish (30-44 cm in length) of the most heavily infected lot (*15*). The present results correspond very well with earlier investigations showing that the fresh herring harbored 2.4-4.0% of the larvae in the flesh at capture (mean: 0.25-0.33 larvae in the flesh of each herring). The separate examination of belly flaps and fillets of herring in the present experiments revealed that approximately 75% of these larvae were situated in the belly flaps. Similar distributions of *Anisakis* larvae, predominating in the hypaxial muscles compared to the epiaxial muscles, have earlier been described for herring (*16*), walleye pollock (*3*), and cod (*13*); and this has considerable economic and public health significance, as the belly flaps may easily be discarded or be restricted to nonhuman usage.

It is well-known (e.g., from many of the above mentioned studies) that the abundance of *Anisakis* larvae increases with age/size of the fish host, indicating a gradual accumulation of nematodes. This was also the case with respect to herring in the present study (Fig. 1). Also, the total number of larvae situated in the flesh increase with fish length, but the relative number (i.e., the percentage of the whole worm burden) decrease (Fig. 1) in both herring experiments, indicating that the individual larva was less prone to penetrate into the flesh of large herring. The multivariate analyses showed that these relationships were statistically significant, but that the total worm burden rather than the size of the fish seems to be responsible.

The possible existence of a postmortem migration from the viscera into the flesh of fish, used for human consumption, may have considerable practical impacts, because the risk to public health increase, and because a possible request for immediate gutting after catch will have large drawbacks for herring fishing practice, as this species normally are caught in very large numbers at one time and are allowed to be landed ungutted. But the present experiments show very clearly that *Anisakis* larvae occur in the flesh already at capture, which implicates that even immediately gutting cannot eliminate the risk from eating raw or inadequately prepared North Sea herring. Furthermore, the present results show that no significant postmortem migration could be demonstrated in stored round herring, which means that immediate gutting of the fish on board may not have any influence on the number of nematode larvae in the flesh.

ACKNOWLEDGMENTS

This study was financed by the commission of the European communities within the frame of the EEC programme in the fisheries sector ('FAR') (project UP-1-18). The authors wish to thank all technical staffs, whose skillful work in the laboratories and on board made the examinations possible. The statistical analyses were carried out under the supervision of Dr. Ib Skovgaard, The Royal Veterinary and Agricultural University, Copenhagen, to whom the authors owe much thanks.

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