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Preliminary notes

Effects of pH values and temperature changes on migration of *Anisakis simplex* DUJARDIN,1845 invasive larvae (Nematoda, Askaridoidea, Anisakidae) in mackerel (*Scomber scombrus*, L.)

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Third stage larvae of the nematode Anisakis simplex *(L3) were isolated by pepsin (artificial) digestion from the hypaxial muscles from Atlantic mackerel (*Scomber scombrus *L.).*

The prevalence of encapsulated larvae on visceral organs was compared with the prevalence of larvae that migrated into muscles.

The relation between the migratory number of larvae and pH in the muscles was analyzed at two temperatures (room temperature and refrigerator), at 24 hour intervals.

The conclusions were based on statistical calculations, which showed that pH and temperature did not have a significant effect on migratory triggers of L3 from the viscera to the hypaxial muscles.

The average percentage of L3 migrated postmortem from viscera is only 4.2 % at room temperature (21°C), and 8.3 % at fridge temperature (4°C).

Key words: *Anisakis simplex,* artificial digestion, migration, L3, pH values, temperature

INTRODUCTION

Anisakis simplex, as a common eurixene parasite of aquatic mammals, is able to cause several pathological effects in men, if ingested with uncooked (pickled, salted or smoked) or slightly cooked (grilled, fried) meat of fish, crustaceans and cephalopods (IGLESIAS *et al.*, 1997).

Anisakiasis may be acute, with a sudden appearance of severe clinical manifestation, or chronic, developing over months or years with few clinical signs or symptoms (ISHIKURA *et al.*, 1993). In both forms, the larvae can induce a strong immune response, leading to allergic disorders in many patients (DEL POZO *et al.*, 1996), ranging from mild urticaria to anaphylactic shock (AUDICANA *et al.*, 1997). Clinical symptoms of acute and chronic anisakiasis include nausea, vomiting, abdominal and epigastric pain, abdominal dilatation by ascites or intestinal gas fitting, movable soft induration and diarrhea followed by normal stools or constipation (ISHIKURA *et al.*, 1993).

The disease has been frequently reported in Japan, although the frequency of infection in Europe is apparently lower than in Asian countries, the number of reported cases has increased during recent years (ARMENTIA *et al.*, 1998).

During the cleaning of fish, before gastronomic preparation, viscera is removed, including also the major number of L3 situated under the organ's serosa. The problem remains, however in the L3 which had migrated postmortem

from viscera, and had crawled in hypaxial muscles. In hake's muscles there been reported 6.6 % of total number of larvae (CATTAN and CARVAJAL, 1984), and 10.9 % in herring's fillets (PICCOLO *et al.*, 1999).

The goal of this study was to determinate the prevalence of anisakid larvae in muscles of Atlantic mackerel, which showed high level of infection in the spring period (unpublished author's data), and to investigate if the temperature and pH changes are basic triggers for migration behavior of *A. simplex* third stage larvae.

MATERIAL AND METHODS

Sixty-four individuals of Atlantic mackerel were sampled just after they were caught in the Adriatic Sea with nets, on the same ship. The average length was 31 cm ± 0.9 in length. The samples were divided in two categories, each with 32 fishes. Every category was divided in four groups, containing 8 fishes. One whole category was held in a closed room, with natural source of air circulation, at the temperature of 21°C. Another category was put in refrigerator, at 4°C. Every day, from each category, one group formed by 8 fishes was taken, eviscerated, and the viscera was taken for larvae identification and counting. The samples were taken in the interval of 24 hours, for three days.

For artificial digestion the whole hypaxial musculature was taken; 10 g of muscle was homogenized adding 200 ml of distilled water. Then 1 ml of 36.5 % HCl was mixed in the solution (pH = 3.5), adding also 1 g of human pepsin. The solutions were put in urine glasses and incubated for 24 hours at 37°C. After incubation, the supernatant was discarded, and L3 swimming vigorously at the bottom were counted.

From the same fish sample, a piece of muscle was taken for measurements with a digital pH meter.

The air temperature was measured daily.

Statistical analyses were carried out with statistical software, using STUDENT's t-distribution and PEARSON's correlation coefficient.

RESULTS

Sampled fish were highly infected with anisakid larvae. The percent of L3 found under the serosis of visceral organs, and the percent of L3 isolated from musculature by artificial digestion is shown in Table 1. Approximately 4.2 % of larvae migrated at 21°C, and 8.3 % at 4°C. By STUDENT t-test the difference between number of L3 from viscera and the number of L3 from the muscles, on different temperature was tested. At 21°C t-value was 27.59 and at 4°C, was 21.47, meaning that the difference between the two samples is statistically significant at the level of significance of 5%.

The average number of L3 isolated from muscles in 24 hours intervals, with correspondent pH values is shown in Table 2.

Table 1: Intensity (mt) and prevalence (%) of Anisakis simplex *L3 on the viscera and in muscles*

		21°C				4°C			
sampling hours	N=8	mt visceral	% visceral	mt muscle	% muscle	mt muscle	% visceral	mt muscle	% muscle
0	group 1	372	99.74	1	0.25	315	100	0	0
24	group 2	314	99.04	3	0.91	348	99.14	3	0.86
48	group 3	351	98.29	6	1.71	329	92.68	24	7.32
72	group 4	387	87.08	50	12.92	360	76.38	85	23.62

Table 2. Number (N) of L3 migrated at corresponding pH values at various hours (h) postcapture

	21°C		4°C	
h	N	pH	N	pH
0	1	5.8	0	5.8
24	3	6.2	3	5.8
48	6	6.3	24	5.8
72	50	6.6	85	5.9

The significance of pH and temperature changes on the number of migrated L3 was tested by correlation coefficient. For both value changes, there was found an insignificant correlation coefficient. The results are given in the Table 3.

Table 3. Correlation coefficient between the number of migrated L3, pH values and hours

	pH	temp.	h
N (21°C)	0.21	0	0.82
N(4°C)	0.47	0	0.96

DISCUSSION

The percentage of anisakid infection in Atlantic mackerel is very high, but contradictionally is poorly reflected on the post-mortem migration of invasive larvae. The reason for that could be found in the fact that this fish has a high fat content and thus has a slow autolytical process. The difference between the number of L3 who had migrated and who stayed on viscera is statistically significant, pointing out that the difference is not accidental. Only a small number of L3 had migrated (an average of 6.3 % at the mean temperature of 12.5°C) in Atlantic mackerel. Very similar results regarding the number of migrated larvae were obtained in other studies (CATTAN and CARVAJAL, 1984). The authors found 6.6 % of larvae in musculature of Chilean hake (*Merluccius gayi)*, and 10.9 % of larvae in herring's fillets (PICCOLO *et al.*, 1999). CATTAN and CARVAJAL (1984) concluded that there is no intravitam migration of larvae in Chilean hake, what is congruent with other reports concerning herring (KHALIL, 1969; DAVEY, 1972).

According to SMITH and WOOTTEN (1975), the viscera – muscular migration of L3 could be explained by physical – chemical changes in the viscera, and by the probable increase of temperature in the interior of the fish.

The temperature values in this study were controlled by keeping the samples on constant temperature. At 21°C, where the autolytical processes were more intense, pH values changed more rapidly then at 4°C. That could present a very unsuitable environment for the larvae, and they could migrate in higher number. However, that was not a proper explanation, because more larvae migrated at 4°C. The most simple reason for that could be found in the optimal pH values for some anisakid enzymes, which help larvae to penetrate in the tissue. The most important are hyaluronidases, a group of hydrolytic enzyme that degrades the glycosaminoglycan hyaluronic acid and glycosaminoglycan chondroitin sulphate A, a basic compound of connective tissue (HOTEZ *et al.*, 1994). Anisakid hyaluronidases work optimally at pH around 4, meaning that better pH value for its catalytic activity was in muscles kept at 4°C (5.8), than at 21°C (6.2 in average). Statistically insignificant correlation between those pH values and migrated larvae, could be explained by the fact that both pH values differed from the optimum. The constant temperature statistically has no effect on the migration behavior. Previous study also found that temperature had no effect on the migration, but pH had a strong influence (CATTAN and CARVAJAL, 1984).

CONCLUSIONS

The results indicate that the migratory behavior is not directly triggered by changes in temperature and pH values, but by the sum of changes in microenvironment conditions, on first place by enzymatic degradation post-mortem, which is catalyzed only at proper pH. Those autolytic processes after the death of the

organism are the most intense in large endocrine and exocrine glands, like liver and pancreas, which are in very close contact with dormant L3 on viscera. The products of biodegradation, like biogenic amines, because of their highly potent biological activity, could be the first triggers for the larvae to initiate a searching for better conditions. In that process they are helped with their own enzyme systems, which once again, can not work if the pH is not at their optimal.

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Efekti pH vrijednosti i promjene temperature na migraciju *Anisakis simplex* DUJARDIN,1845 invazivnih larvi (Nematoda, Askaridoidea, Anisakidae) kod skuše (*Scomber scombrus*, L.)

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SAŽETAK

Invazivna ličinka nematoda *Anisakis simplex* (L3) izolirana je umjetnom digestijom iz hipaksijalnog mišičja skuše (*Scomber scombrus*).

Postotak "uspavanih" ličinki na visceralnim organima uspoređen je sa postotkom ličinki koje su migrirale u mišičje.

Odnos broja migriranih ličinki i izmjerenih pH vrijednosti mišičja analiziran je na dvije referentne temperature (sobna temperatura i temperatura hladnjaka), u vremenskim razmacima od 24 sata.

Zaključci su doneseni na osnovi statističkih metoda obrade podataka, koje su pokazale da pH i temperatura nemaju veći utjecaj na migratorne otponce L3 iz viscere u mišičje.

Prosječan broj L3 koje su migrirale *post-mortem* iz viscere iznosi tek 4.21 % na sobnoj temperaturi (21°C), odnosno 8.28 % na temperaturi hladnjaka (4 °C).