

Research Note

Anisakid Larvae in the Musculature of the Argentinean Hake, *Merluccius hubbsi*

M. VICTORIA HERRERAS, F. JAVIER AZNAR, JUAN A. BALBUENA, AND J. ANTONIO RAGA*

Department of Animal Biology and Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Dr. Moliner 50, 46100 Burjassot, Valencia, Spain

MS 99-308: Received 13 October 1999/Accepted 10 February 2000

ABSTRACT

We report the infection levels of third-stage anisakid larva in the muscle of the Argentinean hake, *Merluccius hubbsi*, in relation to fish size and location in the musculature. The musculature of 42 hake was separated into hypaxial (ventral) and epiaxial (dorsal) parts and surveyed for nematode larvae. Two anisakid species were detected: *Anisakis* sp. (prevalence, 52.4%; mean $\pm$ SD abundance, 1.2 ± 1.7) and *Pseudoterranova* sp. (prevalence, 9.5%; mean $\pm$ SD abundance, 0.2 ± 0.7). Since the fish were gutted after capture, the occurrence of anisakids in the flesh indicates that the worms had migrated into the muscle before capture. The number of *Anisakis* sp. in muscle was not correlated with fish length or weight. Therefore, fish size cannot be used as a predictor of parasite loads in the muscle. Only one *Anisakis* sp. and one *Pseudoterranova* sp. appeared in the epiaxial musculature. The density of *Anisakis* sp. in the hypaxial muscles was significantly higher than that in the epiaxial ones. This suggests that removal of the hypaxial musculature can reduce the risk of anisakid-induced allergies and gastrointestinal anisakidoses among consumers.

The Argentinean hake, *Merluccius hubbsi*, sustains one of the most important fisheries off Argentina and Uruguay. This species has been increasingly imported in Spain, which is the primary port of entry of South American hake products into the European Union market (1). Although there are previous studies of anisakids in *M. hubbsi* (9, 15, 18), none have focused on the occurrence of larvae in the muscles.

The occurrence of anisakid larvae in the musculature of commercial fish entails well-known economic and public health repercussions (4, 16). In addition, recent studies have revealed a new concern for public health, namely, allergy caused by consumption (16) or even manipulation of infected fish (2, 6). Apparently, the antigens produced by anisakid larvae are thermoresistant (16), and, therefore, common prophylactic methods (cooking or freezing) may not prevent allergic reactions among consumers. Therefore, identifying the factors allowing prediction of parasite loads in fish muscles becomes highly relevant.

This article reports the infection levels of anisakid nematodes in the musculature of the Argentinean hake, focusing on two variables that may easily be used to predict anisakid abundance in the muscles: fish size and location (dorsal versus ventral musculature).

MATERIALS AND METHODS

We examined 42 hake captured with fishing lines in the San Matías Gulf (40°50'–42°15'S, 63°45'–65°00'W) from June to No-

vember 1997. The lines were inspected within a maximum of 5 h after deployment, and the fish captured were subsequently gutted on board to prevent postmortem migration of anisakid larvae to the flesh (8, 13, 17). Hake size ranged from 57 to 78 cm (mean $\pm$ SD, 67.5 ± 5.7 cm) and weight from 1139 to 3135 g (mean $\pm$ SD, 1893 ± 521 g). The fish were air freighted and imported fresh by a Spanish supermarket company. The hake were examined for anisakids 3 to 4 days after capture.

The heads and tails were removed from each fish. The remaining musculature, which represented about 35% of the total weight, was separated into the hypaxial (ventral) and epiaxial (dorsal) regions following the horizontal septum. The former is often referred to as the “belly flaps” in the seafood industry (see Romer and Parsons (14) for anatomic details). The nematodes were detected by digestion of the fish musculature at 55°C for 2 h in a solution composed of 1 liter of distilled water, 30 ml of 35% HCl, and 12 g of pepsin from hog stomach. Since the worms were found as third-stage larvae and many anisakid species are formed by complexes of sibling species (see Mattiucci et al (10) and references therein), specimens were identified to the genus level only (3).

The terms *prevalence* (percentage of fish infected with a particular nematode species per number of fish examined) and *abundance* (number of individuals of a particular nematode species in a single fish regardless of whether the latter is infected) are used as defined by Bush et al. (5), but we also use these terms to refer to nematode larvae in specific locations (e.g., prevalence in the epiaxial muscles). Density was calculated as the number of nematodes per kg of musculature.

RESULTS

We found 50 *Anisakis* sp. larvae in the musculature of 22 hake, but only one worm occurred in the epiaxial mus-

* Author for correspondence. Tel: + 34 96 386 4375; Fax: + 34 96 386 4372; E-mail: Toni.Raga@uv.es.

TABLE 1. Infection parameters of *Anisakis* sp. third-stage larvae in the musculature of 42 Argentinean hake, *Merluccius hubbsi*.

Site	Abundance	Prevalence ^a	Density ^b
	Mean $\pm$ SD (median) [range]		mean $\pm$ SD
Hypaxial muscle	1.2 $\pm$ 1.7 (0.5) [0–7]	50.0 (34.9–65.1)	6.1 $\pm$ 9.0
Epiaxial muscle	0.0 $\pm$ 0.2 (0) [0–1]	2.4 (0.0–7.0)	0.1 $\pm$ 0.5
Muscle (total)	1.2 $\pm$ 1.7 (1) [0–7]	52.4 (37.3–67.5)	2.0 $\pm$ 2.7

^a Expressed as percentage, with binomial 95% confidence intervals in parentheses.

^b Number of larvae per kg of musculature.

cles. (See Table 1 for details on infection parameters.) Seven additional larvae of *Pseudoterranova* sp. were detected in the muscles of four hake (prevalence, 9.5%; mean $\pm$ SD abundance, 0.2 $\pm$ 0.7), including one worm in the epiaxial region.

The density of *Anisakis* sp. larvae was significantly lower in the epiaxial muscles than in the hypaxial ones (Wilcoxon test: $z = -4.074$, $n = 42$, $P < 0.001$). The abundance of these larvae in the muscle was not significantly correlated with body length (Spearman rank correlation: $r = 0.191$, $n = 42$, one-tailed $P > 0.1$) or body weight ($r = 0.159$, $n = 42$, one-tailed $P > 0.1$).

DISCUSSION

We are not aware of previous surveys that provide infection data for the muscles of the Argentinean hake, and comparison with other species is difficult because of differences in fish processing. Compared with gutted Chilean hake, *Merluccius gayi*, the prevalence of *Anisakis* sp. in the muscle seems higher in our sample, but the abundance is very similar in both fish species (8).

The lack of correlation between the abundance of *Anisakis* sp. in the muscle and fish size contrasts with observations in other fish species (4, 11, 12). *Anisakis* sp. larvae in fish seem long-lived and accumulate in fish over time (4). However, this does not necessarily imply accumulation in the fish muscles. We suggest that whether anisakid loads in the muscle are correlated with fish size may depend on host-related differences in migration of the larvae from the body cavity into the flesh. (See Roepstorff et al (13) for examples of such differences.)

The fish were gutted on board, and therefore the larvae of *Anisakis* sp. and *Pseudoterranova* sp. had migrated into the muscle before capture. This has been shown in other species (13), including the Chilean hake (8). This involves a potential for allergic reactions among consumers, since about 50% of the muscles were infected and the global density was about two worms per kg of flesh (Table 1). Most worms concentrated in the ventral musculature, up to seven *Anisakis* sp. worms occurred there, whereas few occurred in the dorsal muscles. This pattern differs from that reported in the Chilean hake (7) but is consistent with most data of other fish species (11, 17).

Two practical conclusions can be drawn from this study. First, hake size does not seem a reliable variable to be considered in risk assessment schemes, at least for the size ranges surveyed here. Second, removal of the belly flaps in this species seems a good practice to minimize the

risk of allergy and gastrointestinal anisakidoses. Consequently, despite the popularity of hake steaks among Spanish consumers, fillets represent a safer product and, therefore, a preferable way of commercializing Argentinean hake.

ACKNOWLEDGMENTS

This study was supported by the Comisión Interministerial de Ciencia y Tecnología of Spain (project no. 1FD97–1147), Instituto Valenciano de Estudios e Investigación (project no. 002/077), and European Commission (project no. MCFH-1999-01034). The fish was kindly provided by Mercadona S.A. We thank C. León, N. García, P. Jiménez, and F. Montero for laboratory assistance and comments. F. J. Aznar holds a Research Assistantship from Ministry of Education and Culture of Spain.

REFERENCES

1. Anonymous. 1996. Fisheries and aquaculture in Latin America and the Caribbean: situation and outlook in 1996. FAO Fish. Circ. No. 921.
2. Armentia, A., M. Lombardero, A. Callejo, J. M. Martín-Santos, F. J. Martín-Gil, J. Vega, M. L. Arranz, and C. Martínez. 1998. Occupational asthma by *Anisakis simplex*. J. Allergy Clin. Immunol. 102: 831–834.
3. Berland, B. 1961. Nematodes from some Norwegian marine fishes. Sarsia 2:1–50.
4. Bratney, J., and C. A. Bishop. 1992. Larval *Anisakis simplex* (Nematoda: Ascaridoidea) infection in the musculature of Atlantic cod, *Gadus morhua*, from Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. 49:2635–2647.
5. Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83:575–583.
6. Carretero Aníbarro, P., J. Blanco Carmona, F. García González, M. Marcos Durántez, L. Alonso Gil, M. Garcés Sotillos, R. Pérez Giménez, S. Juste Picón, and M. C. Gutiérrez Ortega. 1997. Protein contact dermatitis caused by *Anisakis simplex*. Contact Dermatitis 37:247.
7. Carvajal, J., P. E. Cattán, C. Castillo, and P. Schatte. 1979. Larval anisakids and other helminths in the hake, *Merluccius gayi* (Guichenot) from Chile. J. Fish. Biol. 15:671–677.
8. Cattán, P. E., and J. Carvajal. 1984. A study of the migration of larval *Anisakis simplex* (Nematoda: Ascarididae) in the Chilean hake, *Merluccius gayi* (Guichenot). J. Fish. Biol. 24:649–654.
9. Mackenzie, K., and M. Longshaw. 1995. Parasites of the hakes *Merluccius australis* and *M. hubbsi* in the waters around the Falkland Islands, Southern Chile, and Argentina, with an assessment of their potential value as biological tags. Can. J. Fish. Aquat. Sci. 52(Suppl. 1):213–224.
10. Mattiucci, S., G. Nascetti, R. Cianchi, L. Paggi, P. Arduino, L. Margolis, J. Bratney, S. Webb, S. D'Amelio, P. Orecchia, and L. Bullini. 1997. Genetic and ecological data on the *Anisakis simplex* complex, with evidence for a new species (Nematoda, Ascaridoidea, Anisakidae). J. Parasitol. 83:401–416.
11. McClelland, G., R. K. Misra, and D. J. Martell. 1990. Larval anisakine nematodes in various fish species from Sable island Bank and

- Vicinity. In W. D. Bowen (ed.), Population biology of sealworm (*Pseudoterranova decipiens*) in relation to its intermediate and seal hosts. Can. Bull. Fish. Aquat. Sci. 222:83–118. (Special issue.)
12. Platt, N. E. 1975. Infestation of cod (*Gadus morhua* L.) with larvae of codworm (*Terranova decipiens* Krabbe) and herringworm, *Anisakis* sp. (Nematoda Ascaridata), in north Atlantic and Arctic waters. J. Appl. Ecol. 12:437–450.
 13. Roepstorff, A., H. Karl, B. Bloemsma, and H. H. Huss. 1993. Catch handling and the possible migration of *Anisakis* larvae in herring, *Clupea harengus*. J. Food Prot. 56:783–787.
 14. Romer, A. S., and T. S. Parsons. 1986. The vertebrate body. Saunders College Publishing, Philadelphia.
 15. Sardella, N. H., and J. T. Timi. 1996. Parasite communities of *Merluccius hubbsi* from the Argentinean-Uruguayan common fishing zone. Fish. Res. 27:81–88.
 16. Smith, J. W. 1999. Ascaridoid nematodes and pathology of the alimentary tract and its associated organs in vertebrates, including man: a literature review. Helminthol. Abstr. 68:49–96.
 17. Smith, J. W., and R. Wootten. 1975. Experimental studies on the migration of *Anisakis* sp. larvae (Nematoda: Ascaridida) into the flesh of herring, *Clupea harengus* L. Int. J. Parasitol. 5:133–136.
 18. Szidat, L. 1995. La fauna de parásitos de *Merluccius hubbsi* como carácter auxiliar para la solución de problemas sistemáticos y zoogeográficos del género *Merluccius*. Com. Mus. Argent. Cienc. Nat. Bernardino Rivadavia Inst. Nac. Invest. Cienc. Nat. Zool. 3:1–54.