



Short communication

Preliminary study on prevalence of larvae of Anisakidae family in European sea bass (*Dicentrarchus labrax*)

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ARTICLE INFO

Article history:

Received 20 November 2007

Received in revised form 19 June 2008

Accepted 3 July 2008

Keywords:

European sea bass

Anisakidae

Zoonosis

ABSTRACT

A total of 561 fresh European sea bass from North-East Atlantic Ocean (F.A.O. zone 27) were examined for Anisakidae larvae detection. It is the first record on the prevalence of Anisakidae larvae in this fish specie. An unexpected high prevalence was found. The prevalence range was between 65% and 85% in relation with the body weight of the subject. The results have an important consequence on epidemiology of anisakidosis and public health risk assessment.

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1. Introduction

Many zoonoses are caused by parasitic helminths of fish, one of these is anisakiasis. Anisakiasis is the human infection with third larval stage of nematodes belonging to the families Anisakidae or Raphidascaridae.

Anisakis and Pseudoterranova are the two genera most associated with anisakiasis. Marine mammals and piscivorous birds are definitive hosts, while aquatic invertebrates and fish are intermediate hosts. In intermediate host, the larvae penetrate the intestine and invade the celomatic cavity or the muscle, where they moult in third stage and become encapsulated. Humans are accidental host for larvae, as the parasite does not develop further in the human gastrointestinal tract. Nevertheless, Anisakidae larvae cause a number of clinical forms, some of which can be very serious. People can incur in this pathology when consuming raw or lightly cooked seafood products. About 20,000 cases of anisakiasis are reported in the literature, over 90% from Japan and the rest from European Union, United States of America, Canada, New Zealand, Chile and Egypt (Chai, Murell, & Lymbery, 2005). The high prevalence of anisakiasis in Japan is clearly correlated to the tradition of consuming raw fish. In Italy, 23 identified cases of human anisakiasis are reported over the period 1996–2007; in all the cases the patient referred the consumption of raw or marinated fish. Anisakiasis is misdiagnosed and underestimated, in fact the diagnosis is often obtained after surgery (Maggi et al., 2000; Montalto et al., 2005; Pampiglione et al., 2002; Pellegrini et al., 2005; Testini,

Gentile, Lissidini, Di Venere, & Pampiglione, 2003; Ugenti et al., 2007). Nowadays, the increase in consumption of raw fish in Italy asks for new epidemiological studies of anisakis in fish species. For this reason, a preliminary epidemiological survey of Anisakidae larvae was carried out on 561 specimens of European sea bass (*Dicentrarchus labrax*) captured off the F.A.O. zone 27. Wild European sea bass is one of the most important species commercialized in Europe, and particularly in Italy, for commercial value and acceptance.

2. Materials and methods

Owing to the absence of prevalence studies in European sea bass, it was adopted a conservative approach. Sample size was estimated using the formula (Daniel, 1999):

$$n = [ZP(1 - P)]/d^2 \quad (1)$$

where n = sample size, Z = Z statistic for a level of confidence, P = expected prevalence (in proportion of one), d = precision (in proportion of one).

In this study, Z value is 2.58 at the level of confidence of 99%. It was assumed a P value of 0.50, because, as suggested by Daniel (1999), if there was doubt about the value of P , it is best to err towards 0.50 that it would lead to a large sample size. It was chosen a precision of 10% (d value 0.10) as a good compromise between an appropriate precision and the resource due to a preliminary study. With these remarks, the resulting n was 166. Stratified random samples of European sea bass from F.A.O. zone 27, with strata defined by body weight, were collected from commercial boxes between March and June 2007. Three weight categories were defined

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Table 1

European sea bass from F.A.O. zone 27 divided in weight category. Sample (N) number of subject examined, positive (N) number of infected subject

Weight categories	1–2 kg	2–3 kg	>3 kg
Sample (N)	334	180	47
Positive (N)	218	153	42
Prevalence (%)	65.27	85.00	89.36

(1–2 kg, 2–3 kg and >3 kg) because it was easier to use commercial categories and the specimen weight is correlated to age. The specimens were so divided: 334 of European sea bass for weight category 1–2 kg, 180 for weight category 2–3 kg and only 47 for weight category >3 kg. The fresh fish was dissected immediately after collection. The fish was dissected by making an incision along the ventral line from the anus to the oral aperture, then the whole body cavity and the abdominal organs were carefully examined to detect the Anisakidae larvae. A subject with one or more larvae was considered positive and potentially harmful for human consumption. Prevalence was defined as the percentage of infected hosts. Yates' chi-square test was used to analyse the association between fish weight and prevalence. Because of its small sample size, it was not calculate the correlation between prevalence and weight for the category >3 kg.

3. Results and discussion

The prevalence in each weight category is reported in Table 1. An unexpected high prevalence was found: the prevalence of Anisakidae larvae in the 1–2 kg European sea bass was 65.27% against 85% in 2–3 kg European sea bass and there was a statistically very significant relationship between fish weight and prevalence of parasites ($P < 0.01$). Owing to the difficulty to collect subjects of more than 3 kg body weight, this sample size group was not enough to determine prevalence with the same precision of the other weight categories, but the obtained result suggested an important infection. The present survey is preliminary, but it provides a number of points of interest, especially because it is the first report of the presence and prevalence of Anisakidae larvae in European sea bass. As the weight increase is correlated with fish age, the observed prevalence increase can be related not only to accumulation of nematodes in the host during its life, but also to the change of diet, in fact large fish preys small parasitized fish, the larvae are able to re-establishing in predator without a moult. Large European sea bass can thus accumulate a very high number of larvae. Italy is the largest European Union market for wild and cultured European sea bass. Considering that European sea bass is marketed whole without evisceration, it is strategic to study in depth the epidemi-

ology of anisakidosis from European sea bass for assuring public health. In fact, the success of control and legislation measures against any parasitic disease is dependent on knowledge of its aetiology and epidemiology (Cox, 1993). From this preliminary work, many important issues are to be raised: the possibility of larvae migration in muscles intra vitam or post mortem; the specie identification of nematodes and their possible survival in European sea bass; the possible differences in prevalence according to fishing zone. While studying these issues, to prevent anisakidosis it is important to adopt good health practices, such as immediate evisceration of European sea bass after capture, without throwing viscera overboard and the precaution of not consuming raw wild European sea bass. After further studies, when the prevalence data in this specie will be confirmed, it will be possible to determine a sample plan for detecting the lots of parasitized fish. The following formula, suggested by Canon and Roe (1982) to determine the sample size to verify parasite free population, could be used:

$$n = [1 - (1 - a)^{1/D}] [N - (D - 1)/2] \quad (2)$$

where n = sample size, a = level of confidence, D = presumed minimum number of infected animal.

Anisakiasis is an important public health problem, this requires to deep its knowledge of each step of the food chain, from fisher to consumer.

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