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Short Communication

Anisakid nematodes prevalence in Chilean hake (*Merluccius gayi gayi*) commercialized in the city of Talca, Chile

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ABSTRACT. The main objective of this work was to determine the presence of anisakid larvae in fresh Chilean hake *Merluccius gayi gayi* coming from the coastal area near the city of Talca, Chile, commonly commercialized by a regional fish supply center located in the same city. Flesh and viscera of 214 fish were analyzed via direct observation, and the prevalence of parasitism was calculated. The results showed a prevalence of 100% and 24.3% in viscera and flesh, respectively. Morphologic characterization of 138 randomly selected larvae was performed via diaphanization technique, observing 106 larvae of *Anisakis* spp., 13 larvae of *Pseudoterranova* spp. and 19 nematodes classified as "unidentified." All larvae here analyzed were viable based on the spontaneous movement ability of parasites. Overall, the high prevalence of fish infected with Anisakidae larvae highlights the importance of taking early intervention actions in order to prevent the occurrence of anisakidosis in the human Chilean population; particularly critical given the current culinary preference for raw fish dishes such as ceviche and sushi, which poses a public health issue.

Keywords: *Anisakis*; *Pseudoterranova*; Chilean hake (*Merluccius gayi gayi*); nematodes; anisakid; prevalence

Anisakidosis disease is caused by any member Anisakidae (Nematoda), a foodborne parasitic zoonosis (Nieuwenhuizen, 2016). In countries such as Japan, Spain, Mexico, Canada and the United States, anisakidosis is considered an endemic disease. In South America, Chile is the country that shows a high prevalence of anisakids in fish (Torres *et al*., 2014), becoming it into a country very susceptible to present cases of anisakiasis in humans. Anisakidae includes 24 genera, three of them with importance in public health: *Anisakis*, *Contracaecum* and *Pseudoterranova* (Jofré *et al*., 2008). Fish are an intermediate host of the L3 infective larvae, which migrate through the digestive wall reaching abdominal organs such as liver, ventral or dorsal muscles, and surrounding connective tissue. Humans are an accidental host of larval stage L3, which in many cases causes gastrointestinal or allergic manifestations (Baird *et al*., 2014; Nieuwenhuizen & Lopata, 2014; Moneo *et al*., 2017). These parasites have been found in several fish species, including the Chilean hake (*Merluccius gayi gayi*) (Jofré *et al*., 2008). This fish species is well distributed along the Chilean coast and is one of the most highly consumed fish of the Chilean people (Quezada & Dresdner, 2014).

In recent times, the consumption habits of the Chilean population have changed. The incorporation of "foreign" dishes such as ceviche, sushi or sashimi, based on raw or undercooked fish, has become a public health issue due to a higher risk of the presence of viable anisakid parasites in food. Based on the fact that Chile is a major consumer of hake, several studies on anisakid parasitosis have been carried out. George-Nascimento (1996) described the populations of parasites in Chilean hake from three major fishing areas of central Chile describing high prevalence (>53%) for *Anisakis* spp., and low prevalence for *Pseudoterranova decipiens* and *Contracaecum* spp. (average 26 and 9%, respectively).

In contrast, another study published by Torres *et al*. (2000) showed a prevalence of 5.9% of *Anisakis* spp., and 23.5% of *Pseudoterranova* spp. in commer-ciallized *M. gayi gayi* in the city of Valdivia, Chile. A

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study by Oliva & Ballón (2002) reported a prevalence of over 80% of *Anisakis simplex* observed in Chilean hake. A study conducted in the extreme south of Chile described the southern hake (*Merluccius australis*) parasitosis, reporting prevalence that averaged 90% for *Anisakis* spp., 49% for *Contracaecum* spp. and 12% for *Pseudoterranova decipiens* (George-Nascimento & Arancibia, 1994). Madrid *et al*. (2016) showed the existence of an increasing prevalence of anisakid parasites among years, since in 2006 was 32.2% compared to 72.5% in 2012 in *Merluccius* spp. commercialized in the city of Concepción, which they had a size and weight on average 40 cm and 500 g, respectively. Scientific data demonstrate the existence of a high risk of human infection by anisakid parasites upon consumption of fish commonly commercialized in Chile. Nevertheless, no studies exist on the prevalence of these parasites in fish destined to human consumption in Talca, Chile. The main objective of this work was to determine the prevalence of nematodes belonging to the Anisakidae in flesh and abdominal organs of fresh Chilean hake coming from the coastal area near Talca, Chile, and likewise to determine the current risk of transmission of this foodborne disease to the population in this city.

In this study, 214 fish were obtained from the coastal area near Talca, Chile, which were commercialized by a regional fish supply center located in the same city. The average weight of fish analyzed was 341.1 g. The individual fish in the study were selected according to a probabilistic sampling assuming the presence of different sizes and weights of fish. The analyses were performed over three consecutive days. Initially, 56 fish were analyzed. A longitudinal cut from the operculum to the tail was performed in order to reach the coelomic cavity and visceral surfaces to obtain larvae. Numerous deep cuts were made to reach the epaxial and hypaxial muscle, and fillets of 5 mm thickness were gotten. The same procedure was performed in all fish in the study on the second and the third days with 53 and 105 fish, respectively. The prevalence was defined as the number of infected fish with at least one larva divided by the total fish examined (Margolis *et al*., 1982). For the identification of anisakid nematodes, a representative sample of L3 larvae was taken using a sample size calculation for finite populations, according to Naing *et al*. (2006). After obtaining the parasites, larvae viability was determined based on spontaneous movement in a Petri dish filled with distilled water. The genus of each larva was determined through the diaphanization technique, which allows the recognition of internal nematode organs (Torres *et al*., 2014). Following the guidelines used by Rello *et al*. (2004) and Quiazon *et al*. (2008), the digestive apparatus and physical characteristics such as size, thickness and color were observed to determinate the classification of anisakid nematodes. Due to technical problems on sample preparations, some individual fish were not identified within a genus; therefore, they were assigned as "unidentified."

Of the 214 fish analyzed in this study, all presented a high number of nematodes (an average of 20 parasites per fish), located mainly on the surface of abdominal organs such as liver, gut, gonads and also free in the coelomic cavity (Figs. 1a-b), which indicates a 100% prevalence in these organs (Table 1). Nematodes were easily observed within the coelomic cavity immediately after the ventral dissection of the fish. In addition, 52 fish presented L3 larvae embedded in the muscle (Fig. 1c, Table 1), most of them found in hypaxial muscle tissue, corresponding to a prevalence of 24.3%. Afterward, 138 L3 larvae from different fishes were used for identification analysis. According to the morphologic characteristics of Anisakidae, three different groups were determined: *Anisakis* spp., *Pseudoterranova* spp. and "unidentified." Thirteen larvae (9.42%) were identified as genus *Pseudoterranova* based on anatomical characteristics such as size, color, appendant glands and form of the tail in its rear end; and 106 larvae (76.81%) as *Anisakis*, which were identified by the digestive apparatus (due to ventricle form) (Fig. 1d). A group of 19 larvae (13.77%) were classified as "unidentified" due to damage during sampling. From all samples analyzed, 100% of larvae presented vigorous movement in distilled water and therefore were considered as viable larvae.

It is well known that the increased risk of zoonosis is constituted by the consumption of raw or undercooked fish muscle. In the present study, we found a prevalence of 24.3% of L3 larvae in the muscle of fresh *Merluccius gayi gayi*. The prevalence in the muscle of *Merluccius* spp. has been reported in other geographic regions of Chile. In Valdivia, Chile, Torres *et al*. (2014) reported the prevalence of *Anisakis* spp. and *Pseudoterranova* spp. in the muscle of *Merluccius australis* showing 8.7 and 4.8%, respectively. Madrid *et al*. (2016) studied the prevalence in the muscle of *Merluccius* spp. reporting prevalence of 32.2 and 72.5% in samples obtained in 2006 and 2012, respectively. Both groups of authors performed a technique called "candling" for larvae identification in muscle. Many factors may contribute to the variation in results between studies such as observation technique used: the depth of cuts or the time elapsed since the evisceration of fish, because it is demonstrated that anisakid larvae migrate to the muscle after the host death (Deardorff *et al*., 1984). Although we performed

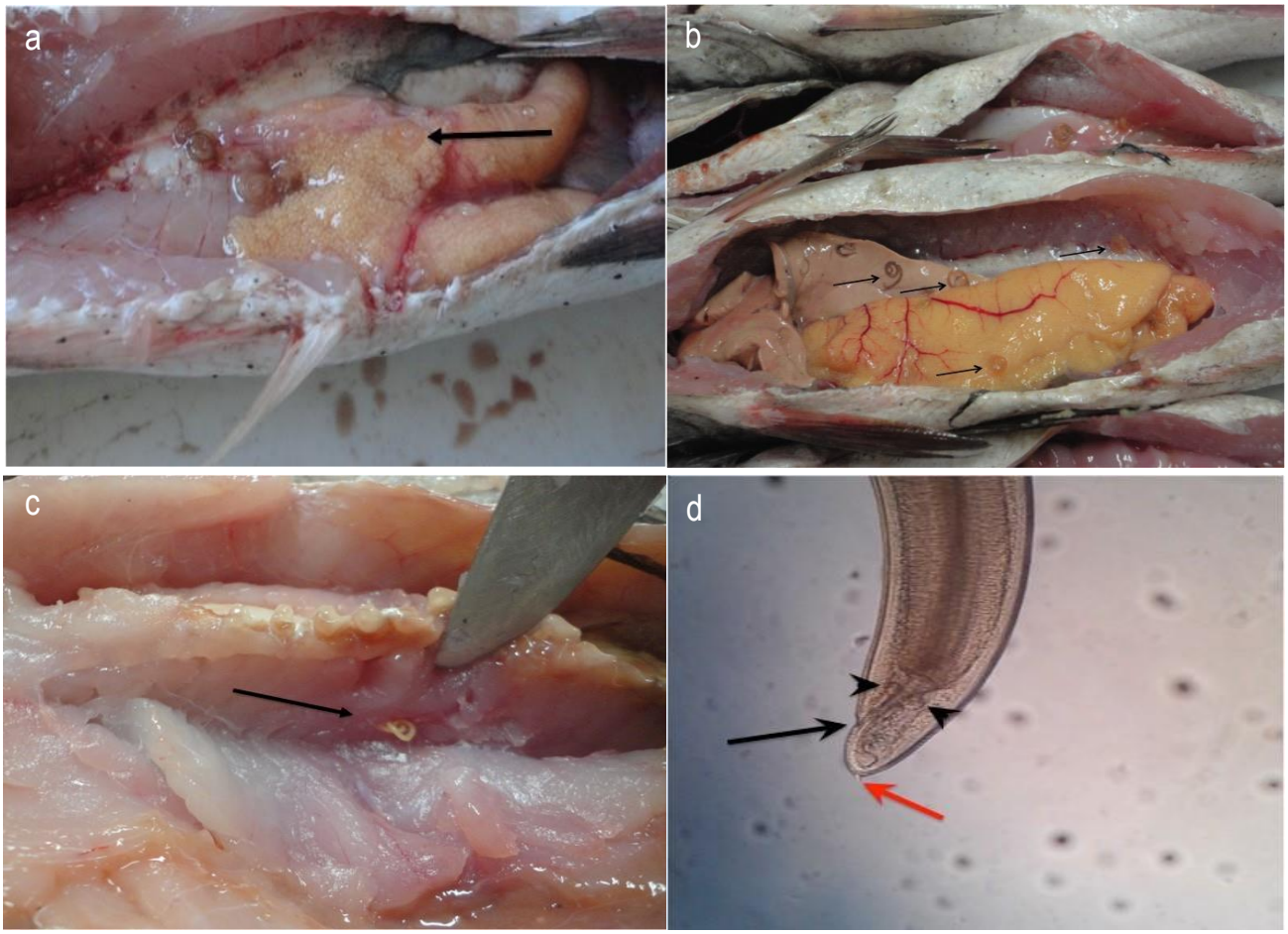

Figure 1. Members of *Anisakidae* in Chilean hake *Merluccius gayi gayi*. a-b) Several larvae in the visceral surfaces (liver, gonads), c) the arrow shows a parasite embedded in fish muscle, d) posterior end of larva (L3) of anisakid. The photograph shows the mucron (red arrow), anal pore (black arrow) and anal glands (arrowheads).

Table 1. Summary of prevalence in analyzed Chilean hake.

Location of parasite	Parasitized fish	Fish without parasites	n	Prevalence (%)
Flesh	52	162	214	24.3
Abdominal organs	214	0	214	100

thin and deep cuts throughout the hypaxial and epaxial musculature of the fish and only analyzed macroscopically these slices, in our study, we could identify embedded parasites mainly in hypaxial muscle tissue, similar to the data observed by Madrid *et al*. (2016).

Using the diaphanization technique, we observed 106 larvae (76.81%) belonging to the genus *Anisakis* and 13 larvae (9.42%) from genus *Pseudoterranova*. These data are similar to those of George-Nascimento (1996) and Torres *et al*. (2014), which describe a lower prevalence of the *Pseudoterranova* genus compared to *Anisakis* spp. However, more updated studies are needed with samples obtained along the coast of Chile to confirm these differences.

A recent study showed the prevalence of *Anisakis* spp. in *Brama australis* in three southern Chilean localities: 16.8% in Lebu, 10.6% in Calbuco and 6.7% in Punta Arenas (Oliva *et al*., 2016). Several data demonstrate the importance of this nematode parasite in public health and the risk of infection due to this seafood-borne disease worldwide, which affects many different fish species destined for human consumption such as: Chilean jack mackerel (*Trachurus murphyi*) (George-Nascimento *et al*., 1983), sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*)

(Serracca *et al*., 2014; Cavallero *et al*., 2015; Molina-Fernández *et al*., 2015), Atlantic mackerel (*Scomber scombrus*) (Pekmezci, 2014), lamprey (*Petromyzon marinus*) (Bao *et al*., 2013), golden kingklip (*Genypterus blacodes*) and snoek (*Thyrsites atun*) (Torres *et al*., 2014). Respecting the salmon farmed industry, Marty (2008) described the presence of anisakid larvae in the viscera of a farmed Atlantic salmon (*Salmo salar*), however, the prevalence described was very low (0.11%) probably because of artificial diets which also contain medications designated for salmon (Sepúlveda *et al*., 2004).

Nowadays, the eating habits of people have changed, with an increase in the consumption of raw marine fish (Bao *et al*., 2017) such as sushi or ceviche. From 1976 to 2005, at least 28 people have been diagnosed with anisakidosis, from which 12 were by consumption of ceviche (Madrid *et al*., 2016). Afterward, at least 5 cases of anisakidosis have been reported in Chile (Celestino *et al*., 2007; Weitzel *et al*., 2015). Torres-Frenzel & Torres (2014) described the presence of viable larvae in commercial hake ceviche in southern Chile, showing a prevalence of 16.7 and 7.1% in examined portions from two cities, Valdivia and Niebla, respectively, highlighting the relevance of this emerging fishborne zoonotic disease.

This study demonstrates the high risk of foodborne infection with anisakid parasites for the Talca city population, mainly through dishes prepared with raw local fish such as ceviche, sushi or sashimi. It is also important to ensure early evisceration of fish followed by optimal storage temperatures under article Nº323 of Chilean Food Sanitary Regulations, which state that all fish-derived products commercialized for human consumption must be free of parasites and their cyst (MINSAL, 2019). Further investigation of the presence of these parasites in dishes prepared in the city of Talca with fish extracted from the coast near the city, as well as to characterize the migration of L3 larvae at different storage times and temperatures is needed.

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