Research Note
Anisakid Parasites in Commercial Hake Ceviche in Southern Chile

PABLO TORRES-FRENZEL1 AND PATRICIO TORRES2*

1Escuela de Ingeniería en Alimentos, Facultad de Ciencias Agrarias and 2Instituto de Parásitología, Edificio de Ciencias Biomédicas, Facultad de Medicina, Campus Isla Teja, Universidad Austral de Chile, Casilla 567, Valdivia, Chile

MS 13-538: Received 12 December 2013/Accepted 5 March 2014

ABSTRACT

The objective of this research was to determine the occurrence of anisakid nematode larvae in hake ceviche sold in restaurants in Valdivia (39°48′S, 73°14′W) and Niebla (39°49′S, 73°22′W), Chile. Between August and November 2012, 78 portions of ceviche were collected (6 from each of the 13 restaurants that sell this product). Each portion was weighted and divided into approximately 36-g samples, which were placed in petri dishes with 0.15 M NaCl. All samples were manually shredded and then examined with a stereomicroscope. Muscles of 41 southern hake (Merluccius australis), a fish sold fresh in Valdivia, also were examined by candling to determine the presence of anisakid larvae. The presence of Pseudoterranova larvae in ceviche sold in Chile was identified for the first time. The pH of ceviche ranges from 4.1 to 4.8, which favors the presence of viable anisakid larvae that tolerate the acid pH similar to that found in the stomach of their mammalian host. No significant differences (P > 0.05) in the prevalence, mean abundance, and mean density of anisakid larvae in ceviche were found between localities. Larvae were detected in ceviche from 3 of 6 restaurants in Valdivia and 4 of 7 restaurants in Niebla. Of the 78 examined portions of ceviche, 21.8% had larvae. The prevalence of viable larvae was 16.7 and 7.1% in the examined portions from Valdivia and Niebla, respectively. In the 41 hake muscle samples from Valdivia, the prevalence (4.9%), mean abundance (0.1), and mean density (0.03) was the same for Pseudoterranova and Anisakis larvae. No inspection processes or pretreatments are currently in place for raw fish to achieve safe conditions for ceviche from restaurants in Valdivia and Niebla.

In humans, the consumption of raw seafood without adequate thermal treatment can be a risk factor for infections due to the transmission of viruses, bacteria, protozoans, and helminths (2). Anisakidosis is a zoonosis caused by nematodes of the Anisakidae family, mainly Anisakis and Pseudoterranova species. This disease usually results from the consumption of raw, cold smoked, or undercooked marine fishes containing the third stage larvae (L3) of these nematodes (2, 5, 26). Anisakidosis is considered an emerging risk for health and food safety (16) because the L3 penetrate the wall of the human digestive tract provoking gastric or intestine illness associated with nausea, vomiting, diarrhea, and severe abdominal pain (26). Larvae can also migrate from the stomach to the esophagus, pharynx, or oral cavity and can perforate the wall of the digestive tract and settle in the abdominal organs (15). L3 sometimes molt into fourth-stage larvae (L4). Ingestion of excretory and secretory parasite products, which are not inactivated by freezing or cooking of the meat, can cause allergic reactions (5, 18).

Adult nematodes live in the stomach of marine mammals (definitive host) such as cetaceans and pinnipeds, which disperse the nematode eggs through their feces. The eggs then develop in the water into L3 that infect crustaceans, squid, and fish (an intermediate or paratenic host), allowing transmission of the parasite to marine mammals and humans (19). Larvae are commonly found in marine fishes, particularly in the viscera and body cavity, where they can be eliminated by evisceration or washing of the fish. However, larvae that have penetrated the muscles may go unnoticed (13). After fish have been captured, L3 in the viscera can migrate to the muscles, adding to the parasite load in the musculature (25).

Human anisakidosis has been reported in 26 countries including Canada, the United States, Mexico, Brazil, Peru, and Chile in the Americas (26, 28). About 97% of 31,575 reported cases of human anisakidosis have occurred in Japan (26). In Chile, about 28 cases of human anisakidosis have been reported since 1976 (28). Of these, 12, 4, and 2 cases were associated with the consumption of ceviche (raw fish cut into pieces and marinated in lemon juice with minced onion, salt, and chili pepper), fried fish, and smoked fish or sushi, respectively (21, 22, 28). Although studies of pathogenic bacteria in ceviche sold in Peru (9, 12) and Mexico (32) have been conducted, no reports of helminth parasites are available.

Complete elimination of anisakid parasites from the muscles of fish is not possible. However, some measures can help mitigate the risk of transmission of these parasites to humans (2). Freezing kills the anisakid larvae, which will prevent infection from consumption of raw food (5, 7, 33). The candling technique, which ranges in sensitivity from 7 to 100% (2, 13, 17), involves visual examination of fresh
fish filets over a transparent surface illuminated from below. In Chile, this technique is required for only exported fish (31). Anisakis and Pseudoterranova larvae have been found in more than 40 species of fish from the Chilean coast (23). Prevalence of Anisakis simplex (sensu lato) infection in the muscles of fish sold in the city of Valdivia was 5.9 to 12.5%, with a mean of one larva per infected fish in Chilean hake (Merluccius gayi), flat fish (Paralichthys microps), and Chilean mackerel (Trachurus murphyi) (29). Prevalences of 23.5 to 70% were recorded for Pseudoterranova decipiens (sensu lato) with a mean of 1 to 2.4 larvae per infected fish in tail hake (Macrouronus magellanicus), red conger eel (Genypterus chilensis), flat fish, and Chilean mackerel (29).

Thus, fish sold fresh are a potential risk for consumers of raw food, especially in the preparation of hake ceviche, as occurred in 8 of 12 cases of Pseudoterranova infection (28).

The objective of this study was to determine whether hake ceviche sold in restaurants in two localities in southern Chile contain viable anisakid larvae and thus pose a risk to consumers.

MATERIALS AND METHODS

Portions of hake ceviche were bought between August and November 2012 from restaurants in Valdivia (39°48’S, 73°14’W) and Niebla (39°49’S, 73°22’W), Chile; Niebla and Valdivia are coastal cities 17 km apart. The two cities together have a total of 90 restaurants; 24 offer ceviche, and 13 of these have hake ceviche available for take away. Three portions of hake ceviche were bought on two occasions with a minimal interval of 7 days from each selected restaurant. Personnel at the restaurants were not informed of the study. The other restaurants offered salmonid (Salmo or Oncorhynchus) or pomfret (Brama australis) ceviche. In wild or farmed salmonids introduced in Chile, no Anisakis or Pseudoterranova infections have been reported (27). In pomfret, only mild Anisakis infection has been reported in viscera and none in muscles (23).

Portions of ceviche in expanded styrofoam containers provided by the restaurants were taken to the Parasitology Institute, Universidad Austral de Chile for analysis. Portions were kept at refrigeration temperature (4°C) for a maximum of 24 h until processed for examination using a destructive method. Before processing, each portion was weighed on a digital balance, and the pH of 14 portions was determined with a surface pH probe. Each portion was divided into approximately 30-g samples and placed in 19-cm petri dishes with 0.15 M NaCl. All samples were manually shredded with tweezers and dissecting needles and then examined with a stereomicroscope.

During the study period, southern hake (Merluccius australis) was sold in both Valdivia and Niebla; fishing for this species is legal from 41°28’ to the south tip of Chile (3). Forty-one specimens of southern hake from markets were examined between October and November 2012. Ventral (epiapsial) and dorsal (hypapsial) muscles of these hake were weighed, and ≤4-mm slices were examined directly using the candling technique following the method of Torres and Puga (31). Anisakid larvae removed from the portions of ceviche and the hake muscles were placed in saline solution under coverslips on microscope slides to determine their viability based on movement. Larvae were fixed in 4% formaldehyde in 0.15 M NaCl (formol-saline) and cleared in lactophenol for morphological identification with a light microscope (29). Larvae were measured using an ocular micrometer.

Prevalence was defined as the number of ceviche portions or infected fish with one or more larvae of a particular anisakid type divided by the number of portions or fish examined, expressed as a percentage. Abundance was defined as the number of larvae in a single ceviche portion or fish. Mean abundance was defined as the total number of larvae in all ceviche portions or examined fish divided by the total number of portions or fish examined. Density was defined as the number of anisakid larvae per 200 g of each portion of ceviche or fish muscle. Mean density was defined as the total number of larvae per each 200 g of each ceviche portion or examined fish divided by the total number of portions or fish examined. The prevalence of larvae in ceviche portions was analyzed with the Pearson chi-square test using EPI DAT 3.1 (OPS-OMS, Dirección Xeral de Saúde Pública da Consellería de Sanidades, Xunta de Galicia, Spain) software. A Mann-Whitney U test was used to compare mean abundance and mean density of larvae in ceviche (24).

RESULTS AND DISCUSSION

The mean weights of the hake ceviche portions among the restaurants in both cities were not significantly different; however, highest weights were up to five times higher than lowest weights among these establishments (Table 1).

Mean characters of 23 larvae were determined: body length, 27.6 ± 0.4 mm (21 to 33.8 mm); maximum width, 1 ± 0.1 mm (0.8 to 1.2 mm); nerve ring, 0.4 ± 0.5 mm (0.3 to 0.5 mm) from anterior end; length of esophagus, 1.6 ± 0.4 mm (0.8 to 2.1 mm); ventriculus elongate, 1 ± 0.2 mm (0.7 to 1.3 mm); intestinal caecum, 0.9 ± 0.1 mm (0.7 to 1.2 mm); and tail, 0.1 ± 0.2 mm (0.1 to 0.2 mm) with a distal spine. For ratios, body length to esophagus was 1:9 to 1:14, body length to ventriculus was 1:23 to 1:42, body length to intestinal caecum was 1:27 to 1:39, body length to tail was 1:154 to 1:256, and ventriculus to intestinal caecum was 1:0.8 to 1:1.4.

All larvae in ceviche were identified as L3 of Pseudoterranova sp., which was detected for the first time in ceviche prepared and sold in Chile. One or more of the ceviche portions from 7 of the 13 restaurants contained Pseudoterranova sp. larvae. Larvae were found in 20% of all the portions examined, but only 11.5% of these larvae were viable (Table 1). Prevalence (χ² = 0.12), mean abundance (z = 0.29), and mean density (z = 0.41) of larvae were similar (P > 0.05) in the portions from both localities (Table 1). Only 7 of 12 and 6 of 22 larvae identified in ceviche from Valdivia and Niebla, respectively, were viable; the rest were dead or had been sliced during the preparation of the ceviche (Table 1). The presence of dead larvae or their products in the ceviche could be a potential risk for the consumer because some of the somatic, excretory, or secretory components are thermostable allergens, as has been reported for A. simplex (sensu lato) (5, 18).

The presence of viable larvae in ceviche was possible because of the tolerance of these parasites to low pH, which was 4.1 to 4.8 in this study; the citric acid in the lemon was a contributing factor. Larvae of Pseudoterranova sp. tolerate these aci pH conditions because they survive and develop into adults in the stomach of marine mammals, which have a pH of 1.5 to 3.5 (14).
TABLE 1. Prevalence, abundance, density, and viability of Pseudoterranova sp. larvae in portions of ceviche sold in restaurants in two cities in southern Chile

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Valdivia</th>
<th>Niebla</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of restaurants</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>No. of portions</td>
<td>36</td>
<td>42</td>
<td>78</td>
</tr>
<tr>
<td>Mean (range) wt of portions (g)</td>
<td>343 (186–670)</td>
<td>345 (128–670)</td>
<td>349 (128–670)</td>
</tr>
<tr>
<td>No. of portions with larvae</td>
<td>9</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Prevalence of larvae (%)a</td>
<td>25 (16.7)</td>
<td>19 (7.1)</td>
<td>21.8 (11.5)</td>
</tr>
<tr>
<td>No. of larvaeb</td>
<td>12 (7)</td>
<td>22 (6)</td>
<td>33 (13)</td>
</tr>
<tr>
<td>Abundance of larvaeceb</td>
<td>0.3 (3)</td>
<td>0.5 (7)</td>
<td>0.4 (7)</td>
</tr>
<tr>
<td>Density of larvad</td>
<td>0.2 (2.3)</td>
<td>0.3 (2.9)</td>
<td>0.3 (2.9)</td>
</tr>
</tbody>
</table>

a Percentage of portions with viable larvae.

b Number of viable larvae is listed in parentheses.

c Mean (maximum) number of larvae for all portions.

d Mean (maximum) number of larvae per 200-g portion.

Based on the candling results, 2 (4.9%) of the 41 hake sold in the Valdivia markets were infected with viable L3 of Pseudoterranova sp. and 2 were infected with Anisakis sp. larvae (Table 2). Six larvae were detected in the 41 fish, three Pseudoterranova sp. larvae and three Anisakis sp. larvae. The estimating mean density of infection for both parasites in all fish examined was 0.05 larvae per 200 g of fish muscle (Table 2). The mean density of infection in only the four infected fish was 0.6 larvae per 200 g of fish muscle (Table 2). In a previous study (29), 1.4 and 6.6 anisakid larvae per 200 g were reported for infected fish based on candling and plate compression techniques, respectively. These larvae including A. simplex (sensu lato), P. decipiens (sensu lato), and/or Hysterothylacium sp. in the Chilean hake, tail hake, red conger eel, flat fish, and Chilean mackerel sold in Valdivia.

In Chile, 89.3% of the cases of anisakidosis have been caused by Pseudoterranova sp. larvae, and only 10.7% of cases have been caused by Anisakis sp. larvae (28). In 2 of the 25 cases of Pseudoterranova sp. infection, larvae penetrated the stomach wall but were in the majority of the cases eliminated through the mouth; similar outcomes have been reported in the United States (28). However, in Japan infection with Pseudoterranova species frequently results in penetration of the stomach wall and severe symptoms requiring endoscopic removal of larvae (4). These differences have been attributed to the geographic variability of Pseudoterranova species or variations in individual responses of the host (4).

The genera Pseudoterranova and Anisakis each include eight species (19, 20), and the L3 of these species can be distinguished genetically but not morphologically. In Chile, Pseudoterranova cattani, whose adults develop in sea lions, and Otaria flavescens and their L3 in Chilean hake, pink cush eel (Genypterus maculatus), flat fish, and corvine drum (Cilus gilberti) have been reported based on morphological and electrophoretic data (10, 11). Unidentified L3 and L4 of Pseudoterranova have been detected in the Chilean dolphin (Cephalorhynchus utripia) and in the black porpoise (Phocoena spinipinnis) (30). A. simplex (sensu lato) has been reported in the black porpoise as L3, L4, and adults (30) and in sea lions as L3 and L4; adult stages and larvae have been identified in the Balaenoptera whales (B. borealis, B. eden, and B. physalis), the bottlenose dolphin (Tursiops truncatus), and the southern right whale dolphin (Lissodelphis peroni) (8). Adults and larvae of Anisakis physeteris (sensu lato) have been reported in the sperm whale (Physeter macrocephalus), and unidentified Anisakis species have been reported in the Chilean dolphin (8, 30).

In conclusion, ceviche sold in Valdivia and Niebla can contain viable Pseudoterranova sp. larvae, which can cause anisakidosis in the consumer. The results of this study, the increasing incidence of human anisakidosis (28), and the fact that 42% of foodborne diseases are caused by consumption of fish or their products in Chile (6) suggest that better sanitary measures are needed to mitigate the risks.

TABLE 2. Anisakid larvae infection in muscles of fresh southern hake (Merluccius australis) from markets in Valdivia, Chile

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Anisakis sp.</th>
<th>Pseudoterranova sp.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of infected fish</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Prevalence of infection (%)a</td>
<td>4.9</td>
<td>4.9</td>
<td>9.6</td>
</tr>
<tr>
<td>No. of larvae</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Mean abundance of infectionb</td>
<td>0.1 (2)</td>
<td>0.1 (2)</td>
<td>0.2 (2)</td>
</tr>
<tr>
<td>Mean density of infectionc</td>
<td>0.03 (0.9)</td>
<td>0.03 (0.6)</td>
<td>0.05 (0.9)</td>
</tr>
<tr>
<td>Mean density of infectiond</td>
<td>0.7 (0.9)</td>
<td>0.6 (0.6)</td>
<td>0.6 (0.9)</td>
</tr>
</tbody>
</table>

a Percentage of the 41 fish examined that were infected with larvae.

b Mean (maximum) number of larvae in 41 fish examined.

c Mean (maximum) number of larvae per 200 g of each fish examined.

d Mean (maximum) number of larvae per 200 g of each infected fish.
of infection. These measures could include (i) regulation of food prepared with raw fish in restaurants by requiring previous freezing and storage at −20 °C or below for 7 days, freezing at −35 °C or below until solid and storing at −35 °C or below for 15 h, or freezing at −35 °C or below until solid and storing at −20 °C or below for 24 h and (ii) sanitary education to create awareness of adequate thermal and freezing processes, including cooking for ≥10 min at 65 °C or at 77 °C in a microwave oven (I).

ACKNOWLEDGMENTS
We thank Carmen Brito, Renate Schöbitz, and Sonia Puga (Universidad Austral de Chile) for their help and advice during this study. We also thank the anonymous reviewers for their helpful suggestions and comments on this manuscript. This work was supported by the Parasite Diversity and Zoonosis Transmitted by Aquatic Organisms program, Dirección de Investigación y Desarrollo (DID 1201002), Universidad Austral de Chile.

REFERENCES