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Detection of Anisakid Larvae in Marinated Mackerel Sushi in Tokyo, Japan

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Raw fish dishes such as sushi and sashimi feature prominently in Japanese cuisine. Consumption of these dishes, however, is known to increase the risk of various parasitoses. Anisakiasis is a classic example and is characterized by an acute onset of sharp epigastric pain usually a few hours after the consumption of seafood. At the end of 2012, the Ministry of Health, Labour and Welfare of Japan amended the Ordinance for Enforcement of the Food Sanitation Act (1), and thus, anisakid nematodes *Anisakis* spp. and *Pseudoterranova* spp. were added to the list of distinct food poisoning agents in the manual of food poisoning statistics. In light of this change, statistics on the incidence and prevalence of *Anisakis* food poisoning became available for the public on the website of the Ministry.

According to the statistics on food poisoning, there were 294 cases of *Anisakis* food poisoning, with 301 infected individuals, between 2013 and 2015, mackerel being the most common source of the infection (62 cases, 21%). Marinated fillets as processed products, rather than sashimi itself (raw fish) caused food poisoning cases related to the consumption of mackerel. One study has also shown the presence of anisakid nematodes, such as *Anisakis simplex* sensu stricto and *Anisakis pegreffii* in processed products of mackerel (2).

In the present study, we focused on *Anisakis* contamination of vinegar-marinated mackerels that were used as part of a sushi topping. Seventy-two samples were purchased from 3 conveyor belt sushi restaurants in Tokyo between April and July 2016 for analysis of the level of contamination with anisakid larvae. The samples were subjected to individual gross examination (Fig. 1), followed by stereoscopic microscopy after slide mounting (Fig. 2). Upon detection, the larvae were isolated from the samples with forceps and/or teasing needles.

We found that 7 of 40 sushi topping samples obtained from restaurant A, where homemade marinated mackerels were used, were contaminated with anisakid larvae. In the 7 contaminated samples, 14 larvae were detected in total (2 larvae per contaminated sample on average). Motility was observed in 3 of the 14 larvae when transferred into physiological saline. All the larvae were subjected to morphological analysis, and 4 of them including all the 3 viable larvae were found to be *Anisakis* type I (Fig. 2). However, because the other 10 larvae were damaged, morphological information was insufficient for typing or species identification. Therefore, DNA samples were extracted using a commercial kit and analyzed by PCR with restriction fragment length polymorphism and/or DNA sequencing of the ITS1

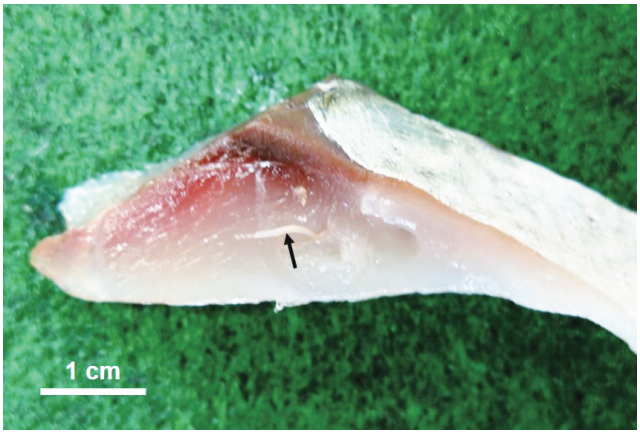

Fig. 1. Anisakid larva in a topping of marinated mackerel sushi (arrow) that is visible on gross examination.

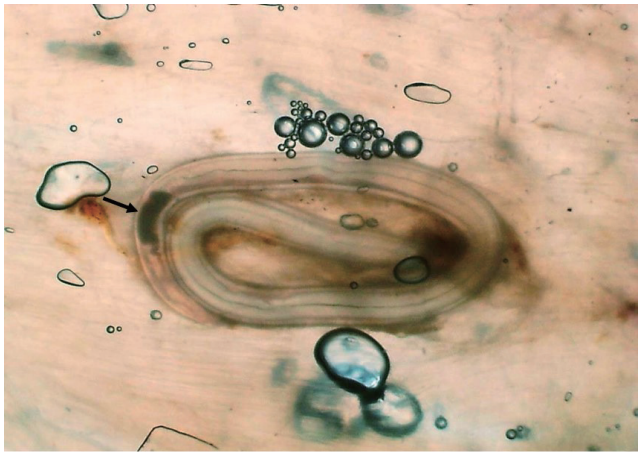

Fig. 2. A mounted slide showing an anisakid larva; the rectangular ventriculus (arrow) is a morphological characteristic of *Anisakis* type I larvae.

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region of the nuclear ribosomal DNA of the parasites (3); all the larvae were identified as *A. simplex* sensu stricto.

Marinated mackerel sushi purchased from the remaining 2 restaurants (32 samples) tested negative for anisakid larvae. One of the 2 restaurants reported having used commercially available marinated mackerel products for the sushi toppings.

Preparation of commercially available marinated mackerels was virtually identical to that of homemade ones. They were then processed for prolonged storage by vacuum-packing and freezing. Anisakid larvae are known to lose their infectivity after freezing at −20°C (or lower) for 24 h (or longer) (4). Therefore, the use of commercially available marinated mackerels and their sushi products safeguards against food poisoning. In other words, homemade marinated mackerels and their sushi products, as sold in grocery stores, are more likely to contain viable larvae that may be infective in humans when consumed. It should be noted that food products that are processed without freezing may cause *Anisakis* food poisoning.

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Conflict of interest None to declare.

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