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Detection of *Anisakis* spp. and residual formaldehyde in Indian mackerel and splendid squid from a fish market in Samut Songkhram Province, Thailand

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Abstract. This study aimed to discover whether *Anisakis* larvae and residual formaldehyde are present in commercially important fresh marine food animals, Indian mackerel (*Rastrelliger kanagurta*) and splendid squid (*Loligo formosana*), obtained from a fish market in Samut Songkhram Province, Thailand. In total, 175 samples of each species were collected in November 2016. Five *Anisakis*-infected fish were found, accounting for 2.86% of the entire sample, but no infected squid was determined. The most infected internal organ was the ovary, which contained 60.9% (n=14) of the discovered larvae. High formaldehyde contamination was observed in both marine animals using a formaldehyde food-testing kit. These results indicate that seafood, such as fish and squid, should be carefully consumed in Thailand and with appropriate food preparation measures.

INTRODUCTION

Parasitic infections in marine animals are important economic issues to fisheries and also in public health. *Anisakis* spp. are parasitic nematodes belonging to the family Anisakidae, order Ascarida (Smith & Wootten, 1978) and are found in the organs of fishes (intermediate host) and marine mammals, such as dolphins and whales (definitive host) (Ferrantelli *et al.*, 2015). Adult parasites range from 2 to 5 cm in length, making them visible to the naked eye; they are found in marine mammals, while cephalopods (particularly squids) and marine fish are hosts of the parasitic larval stage. *Anisakis* spp. cause damage that has an economic impact and also one on human health (Molina-Fernández *et al.*, 2015). Humans are accidental hosts and can be infected by third-stage larvae of this parasite after consuming uncooked marine fish or squids and cephalopods, which leads to anisakiasis (or anisakidosis) and its associated acute gastrointestinal symptoms, such as abdominal pain, diarrhoea, and nausea as well as vomiting (Nieuwenhuizen & Lopata, 2013). In addition, there have been reports of patients with allergies and anaphylactic reactions to this parasite (Ivanoviæ *et al.*, 2017). *Anisakis* infection, which is caused by the consumption of traditional foods containing raw fish, has been reported worldwide, for example in Italy, Korea, Australia, China, Norway, Croatia, and the United States of America (Ivanoviæ *et al.*, 2017), but its prevalence is highest in Japan with roughly 2000–3000 cases reported annually (Yorimitsu *et al.*, 2013).

While there have been no outbreaks of this parasite in Thailand, it is still very important to monitor *Anisakis* infections in marine animals because the number of cases globally is increasing owing to the increasing consumption of exotic food products containing raw fish (Gazzonis *et al.*, 2017). The European Food Safety Authority has recommended continual research into

parasites in fishery products to prevent potential problems (EFSA, 2010). However, and importantly, very little research on this parasite is conducted in Thailand.

Apart from the problems caused by *Anisakis* spp., residual formaldehyde in marine animals must be monitored. Formaldehyde is a volatile toxic aldehyde and considered to be a food contaminant that can be very harmful to consumers; in addition to being toxic to many systems in the human body, it is a possibly carcinogenic. This substance is added to fresh foods to extend their shelf-life by preventing spoilage, and formaldehyde has been found in fish imported from other areas (Siti Aminah *et al.*, 2013; Hoque *et al.*, 2018).

Indian mackerel (*Rastrelliger kanagurta*) and splendid squid (*Loligo formosana*) are widely distributed in the gulf of Thailand and are the most commercially important species in the region. *Anisakis* infection has been described in more than 200 species of fish and 25 species of cephalopod (Molina-Fernández *et al.*, 2015) and is the most frequent parasitic infection in *R. kanagurta* (Hadidjaja *et al.*, 1978). There have been no reports of infection in *L. formosana*, but it has been seen in other squid (Choi *et al.*, 2011). In addition, residual formaldehyde in marine fish is common in many areas (Jung *et al.*, 2001; Siti Aminah *et al.*, 2013).

The present study aimed to determine whether *Anisakis* spp. and residual formaldehyde are present in Indian mackerel and splendid squid from commercially available stock obtained at a fish market in Samut Songkhram Province, Thailand and whether either poses a potential hazard to human health.

METHODS

Fish and squid sampling

A total of 175 samples each of *R. kanagurta* and *L. formosana* were collected on November 2016 from a fish market in Samut Songkhram Province, Thailand (Figure 1). The sample size of 175 was determined based on a total of 320 fish shops using a Krejcie and Morgan table (Krejcie & Morgan, 1970). Fish shops were selected using a table of

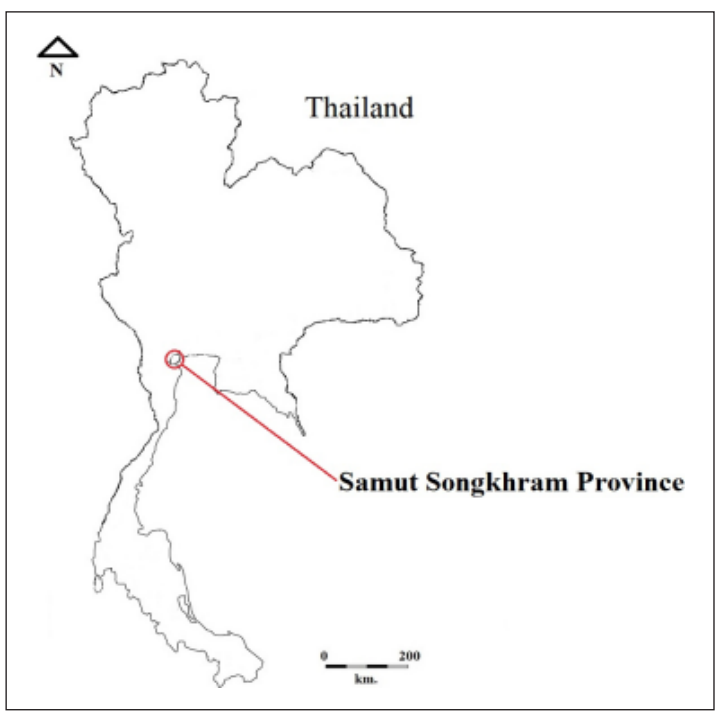

Figure 1. Location of Samut Songkhram Province.

random numbers, and one Indian mackerel and one splendid squid were randomly collected from these shops. Samples were packed into bags (one bag per sample) and transported to the laboratory at the College of Allied Health Science, Suan Sunandha Rajabhat University, Samut Songkhram Education Center, Thailand. The samples were then photographed and re-identified for accuracy according to the criteria established by Collette (1970) for *R. kanagurta* and Natsukari & Okutani (1975) for *L. formosana*.

Parasite detection

After confirming species identities of both the fish and squid, the presence of *Anisakis* third-stage larvae was immediately checked for Nematode larvae were carefully sought in the body cavities and visceral organs of specimens by dissection (Chen & Shih, 2015). Collected larvae were washed with saline solution before species identification. Third-stage *Anisakis* larvae were identified at the genus level based on the morphological characters of Berland (1961) and Smith (1983) using a Nikon AZ 100M stereo microscope (Nikon Corp., Tokyo, Japan). Larvae were then photographed with a digital camera connected to a Nikon Eclipse E600 microscope (Nikon Corp., Tokyo, Japan) under 400× magnification.

Residual formaldehyde detection

Fish and squid tissues remaining from the *Anisakis* examination were employed to detect residual formaldehyde directly after performing the examination using a test kit for formalin in food (consisting of four test bottles containing chemicals that react with formaldehyde) from the Government Pharmaceutical Organization, Thailand (GPO, 2017). One hundred and seventy-five samples each of fish and squid tissue (0.5 g of tissue per sample) were individually added to 45 ml of distilled water and left to stand for 10 min. The tissue was then removed, and distilled water was added to the tested bottle. If a red colour developed within the bottle, the specimen was considered to be positive for residual formaldehyde.

Data analysis

Percentages were utilised for data presentation, including the prevalence of infection in fish or squid, mean intensity (average of infection of parasite among the infected fish or squid), percentage distribution of parasites in internal organs (number of parasites found in each fish organ/total number of parasites in the fish × 100), and percentage of residual formaldehyde in fish or squid (number of formaldehyde-contaminated fish or squid/ total number of fish or squid × 100).

RESULTS

Parasitic infection

A total of 23 *Anisakis* spp. larvae were collected from Indian mackerel specimens but none from squid (Figure 2). Five infected fish were noted, which accounted for 2.9% of the total specimens examined, while there was no infected squid (Table 1).

Anisakis larvae were found in five locations, namely the ovary, serosa of stomach, liver, abdominal cavity, and subcutaneous tissue. The most infected internal organ was the ovary at 60.9% (n = 14; Table 2).

Residual formaldehyde

Formaldehyde contamination was found in both *R. kanagurta* and *L. formosana* tissues. Residual formaldehyde was most common in *L. formosana*, accounting for 57.1% of the total positive samples (Table 3).

DISCUSSION

This study evaluated the presence of *Anisakis* larvae and residual formaldehyde in two commercially important marine animals, Indian mackerel and splendid squid, obtained from a local fish market in Thailand. Five infected Indian mackerel were found in this study (2.9% of the total sample), specifically with the *Anisakis* infection in this species of *Rastrelliger* fish. This result is consistent with the results obtained by *Hadidjaja et al.*

Figure 2. Morphology of *Anisakis* spp. in infected Indian mackerel. Third-stage larvae of *Anisakis* spp. are characterised by the presence of three lips or fairly pronounced labial protuberances with papillae or papilla-like structures, a boring tooth close to the dorsal lip, large excretory gland cells in the anterior region, and a ventriculus between the oesophagus and intestine.

Table 1. *Anisakis* infection in Indian mackerel and splendid squid

Species	Number of specimens examined	Number of infected fish or squid	Mean intensity	Prevalence of infection
R. kanagurta	175	5	4.6	2.9
L. formosana	175	0	0	0

S.D., standard deviation

Table 2. Distribution of *Anisakis* parasites in the visceral organs of Indian mackerel

Visceral organs	*Anisakis* larvae found in Indian mackerel	
	Number of parasites	Percentage distribution of parasites in organs
Ovary	14	60.9
Serosa of stomach	2	8.7
Liver	2	8.7
Abdominal cavity	3	13.0
Subcutaneous tissue	2	8.7
Total	23	100

Table 3. Number of residual formaldehyde-positive fish or squid

Type of marine animals	Number of positive specimens	Percentage of positive specimens
R. kanagurta	9	42.9
L. formosana	12	57.1
Total	21	100.00

(1978) who located *Anisakis* larvae within *R. kanagurta* in the waters around the Seribu Islands, near Jakarta, Indonesia. In addition, Ilahude *et al.* (1978) and Ilahude (1980) found infected *R. brachysoma* in Indonesia; *R. brachysoma* is another major economic fish of Thailand. *R. kanagurta* is widely distributed in the seas of the Indo–West Pacific, from South Africa and the Red Sea to Samoa and northern to southern Japan (Amin *et al.*, 2014). The infected internal organs in the present study were the ovary, stomach, liver, abdominal cavity, and subcutaneous tissue; this is in line with the findings of Dadar *et al.* (2016), who found *Anisakis* larvae in these visceral organs. In the present study, we did not find any parasitic infection in *L. formosana*, which is in agreement with Choi *et al.* (2011), who described a very low infection rate in *L. bleekeri* owing to its small size, hard flesh, and narrow body cavity. The importance of *R. kanagurta* is based on its potential as a food organism. Thus, people may become infected by this pathogen after eating raw or minimally cooked fish, potentially suffering from acute gastro-intestinal symptoms as a result.

We also found a high proportion of formaldehyde contamination in both *R. kanagurta* (42.86%) and *L. formosana* (57.14%). Formalin features approximately 40% formaldehyde and is added as preservative to prevent spoilage of fresh foods and seafood, in particular (Hoque *et al.*, 2018). Formaldehyde is hazardous to humans and animals and is both acutely toxic and chronically toxic as well as carcinogenic (IARC, 2004). The formaldehyde detection results in the current work are consistent with those from another survey (Siti Aminah *et al.*, 2013). One reason that this substance is found at high levels is because it is used by fishing boat operators who are at sea for approximately one week at a time and therefore require a method to prevent spoilage of their catch until they can be sold in markets.

As the present study has demonstrated that level of infected fish was very low, and the presence of *Anisakis* is not a problem, though formaldehyde contamination in splendid squid (57.1% of the total positive samples), the risks of food-borne anisakiasis and formaldehyde contamination should be understood by the public, and health agencies should be aware of these dangers. These results indicate that seafood, such as fish and squid, should be carefully prepared to avoid the consumption of raw or poorly cooked, marinated, or salted marine fish or squid (Ivanoviæ *et al.*, 2017). Infection can be prevented by either cooking or, if consumed raw, freezing to 35°C or below for 15 h or 20°C or below for seven days as recommended by the US Food and Drug Administration (Ivanoviæ *et al.*, 2017). In addition, washing these marine animals with vegetable or meat cleanser prior to consumption will reduce residual formaldehyde contamination.

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