ANISAKIDS IN MARINE FISH FROM THE COAST OF CHON BURI PROVINCE, THAILAND

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Abstract. A total of 1,600 specimens, consisting of 16 different species of marine fish, were dissected and examined for anisakid larvae and adults in visceral organs, abdominal cavity, and muscles. One species of adult-stage nematode was found in two of 16 species of marine fish studied, *Johnius carouna* and *Dendrophysa russelli*. No anisakid larvae (third-stage) was found in any of the 16 species of marine fish studied. Morphological study of the adult-stage nematode showed similar morphology to *Anisakis simplex*. We found that the nematode adult recovered from the marine fish differed from other anisakids in morphology, life cycle and locality of infection in the fish. The anisakid adults recovered were ovoviviparous or larviparous, but not oviparous as is seen in most other anisakids. The intensity and prevalence of nematode infection in *Johnius carouna* were 2.4 and 31.7%, respectively, and in *Dendrophysa russelli* 3.9 and 87.5%, respectively.

INTRODUCTION

Anisakid nematodes or *Anisakis* spp have been implicated in causing human infections by consumption of raw or undercooked seafood. Humans usually act as accidental hosts, as the ingested anasakid larvae are unable to multiply or develop into adult worms in humans. Infections by third-stage anisakid larvae are known to be a causal agent of the human disease called anisakiasis or anisakiosis (Van Thiel, 1962). Symptoms of infection can lead to more severe cases with acute abdominal pain, much like acute appendicitis accompanied by nausca. Anisakid infections are frequently reported in Japan and other Asian countries where consumption of raw fish is high.

Anisakid larvae (*A. simplex*) have been recorded world-wide in approximately 200 fish species (Smith and Wooten, 1978; McClelland *et al*, 1990) and in 25 Cephalopod species (Hochberg, 1990; Abollo *et al*, 2001). Anisakid adults usually inhabit the stomachs of sea mammals, such as small whales, dolphin and seals, which pass eggs with feces into the ocean. The released matured eggs hatch and the larvae are then ingested by tiny crustaceans, which become infected and are in turn eaten by fish and squid. The larvae mature in the viscera and muscles of the fish until becoming infective third-stage larvae. Marine mammals are then infected by eating infected fish.

Anisakid larva type I and *Terranova* larva type B have been recorded from marine fish in the Philippines, Indonesia, Malaysia, and Thailand (Cabrera, 1968; Hadidjaja *et al*, 1978; Loeng, 1980; Bhaibulaya, 1981). In Thailand, Bhaibulaya (1981) reported ascaridoid nematode larvae found in marine fish from the gulf of Thailand, and until recently only few or rare cases of anisakids infection were ever reported in Thailand. With the increasing trend and popularity of consuming sushi and sashimi among the younger generation, the incidence of anisakiasis is expected to increase significantly in the Thai population in the future.

MATERIALS AND METHODS

Fish host samples

A total of 1,600 specimens from 16 different species of marine fish were collected and used in this study. The species of marine fish were: *Johnius carouna*, *Dendrophysa russelli*, *Eutheronema tetradaetylum*, *Muraenesox cinereus*, *Scatophagus argus*, *Drepane punctata*, *Nemipterus hexodon*, *Scolopsis taenioptenus*, *Rastrelliger neglectus*, *Thunnus tonggol*, *Cynoglossus cynoglossus*, *Hemibagrus nemurus*, *Scomberomorus commersoni*, *Lates calcarifer*, *Liza subviridis*, and *Dussumieria elopsoides*. All fish samples were collected between October 2004-April 2005 from commercial landings in Tambon Ang Sila, on the sea coast of Chon Buri Province, Thailand. All fish

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were collected, placed on ice and transported to the laboratory for weight (total body weight, BW) and length (total body length, BL) measurement, followed by morphological identification.

Parasite sample examination

Marine fish were dissected carefully and examined thoroughly for anisakid larvae in the stomach, intestine, visceral organs, abdominal cavity, and muscles. Muscles were digested by pepsin. The larvae were removed from the surrounding host tissue with the aid of a stereomicroscope, counted, and the sites of infection noted. Morphological examinations were carried out with fresh and fixed larvae. After washing in physiological saline, all fresh specimens were examined directly using a stereomicroscope and light microscope. Specimens were prepared and stained with Semichon's carmine, dehydrated in an alcohol gradient series, and mounted in permount balsam.

Identification

Identification was based on comparison of the morphological characteristics of larval types of anisakids. Identification of anisakid nematodes to species and determination of developmental stage were based on morphology of the digestive tract, the shape and the presence of the boring tooth or three lips on the anterior end, the position of the excretory pore, the length and shape of the ventriculus, the presence, length and position of the anterior intestinal cecum and posterior ventricular appendix, and the shape of the postanal tail with a typical terminal mucron (Koyama *et al*, 1969; Berland, 1989; Ishikura and Namiki, 1989; Anderson, 2000; Shih and Jeng 2002, Shih, 2004).

RESULTS

Only 2 species of marine fish, *J. carouna* and *D. russelli* were identified as harboring adult- stage nematodes. Adult female worms were found in *J. carouna* and *D. russelli* at rates of 92.31 and 83.46%, respectively. The results showed higher parasitic infection for *D. russelli* than *J. carouna* (Table 1). Interestingly, only one type of adult nematode was recovered from both species of marine fish, and no anisakid larva was found or identified from either species or from any marine fish collected in this study. Most of the worms recovered were alive and active.

Morphological examination showed that, in general, the morphology of all adult specimens examined by light microscope closely resembled *A. simplex*. Adult-stage specimens had a cylindrical and slender shape attenuated at both ends. The males and females were 0.5 to 1 mm in width and respectively 9 to 24 m;. Adult female anisakids can be 2 - 3 times larger than adult males. A boring tooth was seen at the head; no head bulb and no proboscis was observed (Fig 1). The uterus of the mature adult female was usually characterized with many eggs and larvae

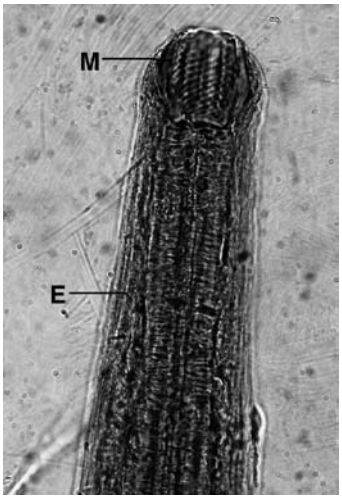

Fig 1- Mouth part and esophagus of adult anisakid from marine fish, *J. carouna* and *D. russelli*. E = esophagus; M = mouth part.

Table 1
Intensity and prevalence of parasitic infections in marine fish.

Marine fish	No. of fish	No. of infected fish	Prevalence of parasitic infection (%)	No. of adult male worms (%)	No. of adult female worms (%)	Intensity of parasitic infection
Johnius carouna	120	38	31.7	7 (7.7%)	84 (92.3%)	2.4
Dendrophysa russelli	120	105	87.5	67 (16.5%)	338 (83.5%)	3.9

Prevalence of parasitic infection = no. of infected fish/ no. of all fish
Intensity of parasitic infection = no. of worms/no. of infected fish

(Fig 2). The cuticle parts were transversely striated and translucent. The worm's esophagus had a long anterior muscular part and an oblong ventriculus with oblique esophago-intestinal junction. No intestinal cecum was observed. The tail was short and with a mucron structure clearly seen (Fig 3). The tails of the adult males were clearly identified with the presence of a spicule and copulatory bursa (Fig 4).

The distribution and locality of infections by anisakid nematodes in the fish bodies showed that the majority of worms found from both species of marine fish were in the intestine, followed by the liver and stomach (Table 2). The highest percentage of worms was found in the intestine, with 64.8% and 80% for *J. carouna* and *D. russelli,* respectively. Other body parts of both marine fish, such as the fins, lungs, and eggs in the uterus, were also infected and no post-mortem migrations of adult worm adults into the flesh was observed, because they cannot migrate to the fleshy parts of the fish. Observation of wide distributions indicates the ability of these worms to migrate into different locations of marine fish organs.

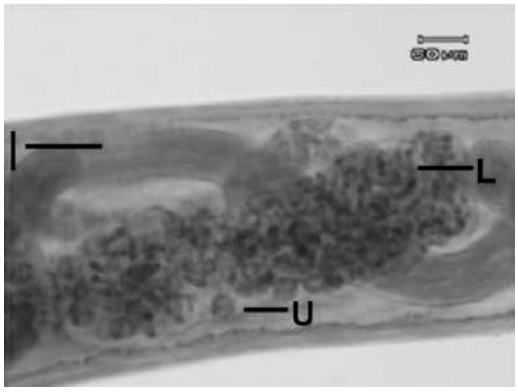

Fig 2- Larvae in uterus of anisakid adult female. I = intestine; L = larvae; U = uterus

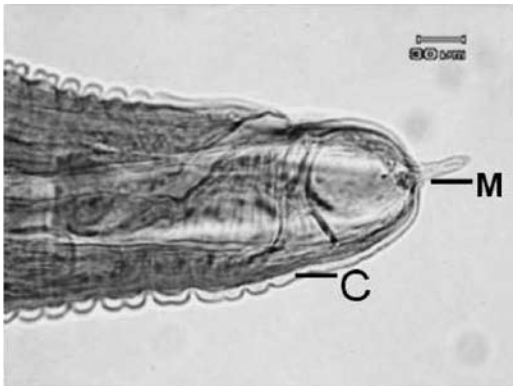

Fig 3- The shape of the posterior end with a typical terminal mucron presented in adult female anisakid. C = cuticle; M = mucron.

DISCUSSION

Morphological examination showed that adult specimens resembled *A. simplex* (Shih, 2004). However, the parasites we examined had a mucron on the tail of the adult female, whereas in *A. simplex*, the larvae of both sexes have a mucron, but adults do not. Moreover, the copulatory bursa has not been noted in male *A. simplex* and *Pseudoterranova ceticola* (Abollo and Pascual, 2002). Also, the uterus of the female contained many eggs and larvae at different stages of development.

In contrast to the well-documented life cycles of anisakids, all larval nematodes recovered from cephalopods (Hochberg, 1990) and fish (Smith and Wooten, 1978; Valdiserri, 1981; McClelland, 1990; Koie, 1993; Stromnes and Andersaen, 1998; Abollo *et al*, 2001; Shih, 2004) were identified, while the anisakid adults usually inhabit the stomachs of sea mammals, such as small whales, dolphin and seals, which act as definitive hosts. Our study clearly showed the adult anisakid nematode can be found in two different species of marine fish (*J. carouna* and *D. russelli*) taken from the coastal seawaters of Chon Buri Province, Thailand. This finding has never been reported before and may be the first ever reported case in Thailand where anisakid adults were found in marine fish.

This investigation found only adult nematodes, primarily not only in the intestine, but also the liver

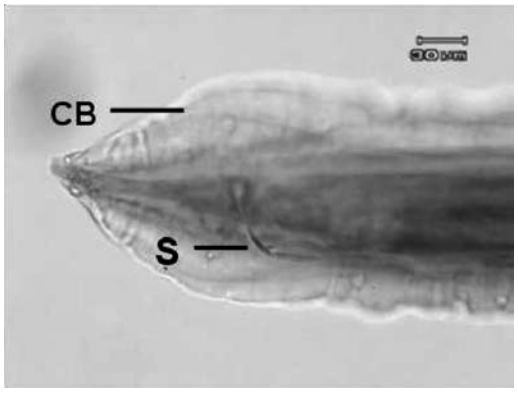

Fig 4- Characteristic of the tail of adult male anisakid. CB = vopulatory bursa; S = spicule.

Table 2
Distribution of worms in each organ of marine fish, *J. carouna* and *D. russelli*.

Organ of fish	Distribution *J. carouna*	Distribution *D. russelli*
	No. (%)	No. (%)
Fin	2 (2.2)	16 (4.0)
Liver	13 (14.3)	11 (2.7)
Stomach	12 (13.2)	34 (8.4)
Intestine	59 (64.8)	324 (80.0)
Lung	1 (1.1)	18 (4.4)
Eggs in uterus	4 (4.4)	2 (0.5)
Total	91 (100)	405 (100)

and stomach. In contrast, the distribution of anisakid larvae in fish body in Galicia (Abollo *et al*, 2001) showed that in some species of marine fish, such as in *Scomber scombrus* and *Trachurus trachurus* species, the majority of anisakid larvae were in the viscera (≥58.9% of the total worm burden). In another species of marine fish, *Micromesistius poutassou*, post-mortem migrations of anisakid larvae from the body cavity to the skin, without encysting in the fish flesh was observed and reported (Abollo *et al*, 2001).

Until recently, it has been well documented that anisakid nematodes are oviparous. However in contrast to the reported characteristic life cycle of anasakid adults elsewhere, our observations shows that the nematodes adults recovered from both marine fish, *J. carouna* and *D. russelli* in coastal seawaters of Chonburi Province, Thailand, were ovoviviparous or larviparous. This finding of both eggs and larvae has never been reported before. Moreover, the anisakid-like nematodes found in the stomachs and intestines of these marine fish were in an advanced developmental, or even reproductive stage (observation of the intestine), therefore indicating that these marine fish (hosts) could act as the definitive host.

More study using other advanced tools, such as scanning electron microscopy, molecular methods, and phylogenetic analysis, are needed for further identification and confirmation of the species observed in our study.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Faculty of Science, Burapha University. The authors would like to express sincere thanks to Assis Prof Dr Ronald A Markwardt, Faculty of Public Health, Burapha University, Assist Prof Chalit Komalamisra, Faculty of Tropical Medicine, Mahidol University, Assoc Prof Dr Paiboon Sithithaworn, Assoc Prof Dr Samarn Tesana, Department of Parasitology, Faculty of Medicine, Khon Kaen University, for their valuable suggestions, guidance and criticism. We thank Ms Ratima Kruvanjaroen, Institute of Marine Science, for identification of marine fish, Mr Peremsak Ieumjumrus and Ms Prapapun Ieumanun for helping to collect the samples.

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