Veterinary Parasitology 171 (2010) 277–285

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

# *Anisakis simplex* (Nematoda: Anisakidae) third-stage larval infections of marine cage cultured cobia, *Rachycentron canadum* L., in Taiwan

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ARTICLE INFO

*Article history:*
Received 23 December 2009
Received in revised form 8 March 2010
Accepted 15 March 2010

*Keywords:*
*Anisakis simplex*
Cage farm
Cobia
*Rachycentron canadum*
Third-stage larvae

ABSTRACT

The first confirmed case of *Anisakis simplex* infection of the marine cage cobia, *Rachycentron canadum* (L.), was recorded in Taiwan. The case investigation revealed the presence of third-stage larvae (L3) in either the stomach lumen or abdominal cavity of the cobia but never within the musculatures. Larvae were mainly encapsulated in the peritoneal mesentery on the outer surface of the stomach wall and occasionally on the liver surface. Part of the diet fed to the cobia includes chopped raw fish, and of these, seven species were found to harbor these larvae (as paratenic hosts), indicating that these particular fish might be the larval sources for this infection. To illustrate the course of infection and distribution of this parasite inside cobia, both juvenile and adult cobia were experimentally infected with live L3 by oral transmission. The prevalence of infection reached 100% at the end of all trials. The course of the infection was assessed after necropsy by histological and ultrastructural observations. *A. simplex* L3 recovered from various locations within juvenile cobia at different post-infection (p.i.) times were at the L3 stage and did not grow significantly. The L3 either adhered to or penetrated into the gastric mucosa of cobia by 2 h p.i. By 25 d p.i., many were trapped within the submucosa and encapsulated by fibroconnective tissue. This phenomenon was more apparent in adult cobia, such that 37.5–86.0% of the injected L3 were primarily found encapsulated within the gastric submucosa. Based upon a PCR-RFLP assay, the larvae encountered in this study were identified as having a recombinant genotype of *A. simplex* sensu stricto and *A. pegreffii*. Based upon the results of this study, strategies to ensure the safety of seafood manufactured from cobia and to prevent the potential risks of anisakiasis or allergies risk to consumers were suggested.



## 1. Introduction

When a given fish species is reported as one of the paratenic hosts in the life history of *Anisakis simplex*, two aspects of the situation become important to humans: esthetics and public health (Schmidt and Roberts, 2000). The first aspect relates to the disgust experienced by persons who find worms in the fish meals that they are preparing or eating. The latter and more-important aspect concerns the pathological conditions caused by penetration or migration of the *A. simplex* larvae, which is referred to as anisakiasis in humans. Acute gastric anisakiasis, which is the most frequent clinical entity, is accompanied by symptoms of epigastric pain, nausea, and vomiting. In addition, the allergenic potential of *A. simplex* has recently been recognized (Moreno-Ancillo et al., 1997). Although patients typically have recurrent hypersensitivity symptoms after the intake of various fish, they have neither

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doi:10.1016/j.vetpar.2010.03.023

skin prick test (SPT) responses nor specific IgE for the implicated fish. Since the involvement of an IgE-mediated response that is specific for *A. simplex* has been proven, an *A. simplex* allergy has been described as a distinct clinical entity that is independent of parasitism (Daschner et al., 2000).

Cobia, *Rachycentron canadum* (Linnaeus), is a highly prized, commercially important fish found worldwide from the Indo-Pacific waters to the southern Atlantic Ocean (Bunkley-Williams and Williams, 2006). It has been farmed in Taiwan since the early 1990s, and currently, nascent cobia aquaculture operations can be found throughout Southeastern and Eastern Asia as well as in the Gulf of Mexico, the Caribbean Sea, and the United States (McLean et al., 2008). Many other nations are presently considering adopting cobia as a new species for aquaculture. The interest in the species has increased due in large part to its many excellent characteristics, including good growth, with production 6-kg live weight fish being possible over a year-long production cycle (Liao et al., 2004; McLean et al., 2008). Additionally, cobia is regarded as having the highest potential for net cage aquaculture, and around 80% of marine cages are presently devoted to cobia culture in Taiwan (Liao et al., 2004).

A dormant threat of anisakid nematode infection to the cage farming industry in Taiwan had been pronounced, as heavy infections with *Hysterothylacium aduncum* and many other nematodes, such as *A. simplex*, *Porrocaecum decipiens*, and *Raphidascaris trichiuri*, had been commonly discovered in cutlass fish and other wild fish around Taiwanese waters (Shih and Jeng, 2002; Shih, 2004). Furthermore, as early as the 1960s, *Anisakis*-type larvae were recorded from both chondrosts, including *Squalus megalops*, *Galeorhinus japonicus*, and *Raja hollandi*, and a few teleosts, including *Caranx* (*Selaroides*) *leptolepis*, *Rastrelliger kanagurta*, *Scomber japonicus*, *Mene maculata*, *Sparus macrocephalus*, *S. sarba*, and *Priacanthus cruentatus*, taken around Taiwan and its offshore islands (Myers and Kuntz, 1967). These concerns became a reality when troubles with the nematode infection occurred in cage-farmed cobia in the Penghu archipelagos, which are located in the Taiwan Strait, where cage cultures are centralized.

This study investigated several aspects: (1) the first case of *A. simplex* infestation in cobia from Taiwan; (2) the possible larval sources for this infection; and (3) the distribution of *A. simplex* larvae inside juvenile and adult cobia by means of experimental infections.

## 2. Materials and methods

### 2.1. Field investigations of cobia infection and A. simplex larvae sources

Interviews with the cobia cage farm where the nematode infections occurred revealed the practice of occasionally feeding cobia with chopped fish before harvest. From March to May 2004, six specimens of *R. canadum* (body length 60 cm; body weight 3 kg) from the cage farm where chopped raw fish were frequently fed to cobia, along with specimens of the local raw fish, were collected and brought to the laboratory for careful examination. The body cavity, gastrointestinal tract, belly flaps, and musculature of the cobia were examined for larval *A. simplex* and other metazoan parasites under a stereomicroscope. The raw fish were identified, measured (body length), and examined the same manner. Nematode larvae were fixed, stored in 70% alcohol, and cleared in glycerin before examination. Third-stage larvae (L3) of *A. simplex* complex were first identified by the following morphological characters: (1) the shape and the presence of the boring tooth; (2) the shape of the tail and the presence of the mucron; (3) the presence of a long ventriculus and the absence of either intestinal cecum or ventricular appendage; (4) the position of excretory pore (Smith, 1983; Anderson, 2000; Shih, 2004).

### 2.2. Experimental infection of cobia

Two groups of cobia at different stages were used for experimental infection trials. Juvenile cobia were used to monitor the early migration of the L3 inside this host and the time course of the short-term migration. Adults were used to reveal the larval distributions over a long-term time course. For the first trial, twelve juvenile cobia (each weighing approximately 140 g) bred by the fish farm at the National Taiwan Ocean University in Keelung, Taiwan and never fed with raw fish were transferred to the laboratory. The fish were acclimated for a week in a 250-L tank. The tank was aerated and supplied with seawater, and the temperature was maintained at $26 \pm 1\,^{\circ}$C using a heating device throughout the trial. The fish were fed a mixture of commercial white fishmeal and water that was freshly prepared to be small enough to fit the mouth of the fish. They were fed twice a day at a daily feeding rate of 3% of total body weight. After acclimation, nine fish were orally administered live *A. simplex* L3 through their diet, and the three remaining fish were used as controls. Each fish was infested with a total number of 30 L3. The L3 used in this trial were collected from the gastrointestinal tract lumen of the cutlass fish, *Trichiurus lepturus*, from commercial catches. All of the cobia were successfully fed, with no observed regurgitation. At 2 h, 24 h, and 25 d after exposure to the larvae, three fish were randomly sampled, and their gastrointestinal tracts, body cavities, and muscles were examined. Larvae were collected, identified, and measured for body length by using a calibrated ocular micrometer. The data were analyzed using Duncan's new multiple range tests to identify the statistical difference.

The second group included eight cobia adults (each weighing approximately 6 kg) from a cage farm in Penghu, Taiwan where raw fish were never fed to cobia. After acclimatization, six of them were fed as described above over three consecutive days, so that each fish was infested with a total number of 200 L3. The remaining two fish served as controls. Cobia were sacrificed and examined for larval *Anisakis* distributions 102 days post-infection (d p.i.). Each fish was filleted and candled. The muscle surrounding the body cavity was carefully examined by simultaneously employing two tweezers and tearing the muscle into individual fibers. The viscera were examined under a dissecting microscope. The stomach walls were cut into pieces and