Vol. 35: 31–36, 1999 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published January 7

Natural mass infection by heterophyid metacercariae in aquacultured Japanese eel in Taiwan

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ABSTRACT: A natural mass infection of heterophyid metacercariae in aquacultured Japanese eel *Anguilla japonica* in Taiwan was observed. Of the 28 000 adult eels in 2 ponds, about 25 000 (90%) showed swollen, cloudy and white eyes. Although morbidity was about 90%, there was no mortality among the affected eels. Histopathological sections showed edema and hemorrhage of the eye. Numerous metacercariae were observed in the muscle tissues around the eyeball, the subcutaneous tissue and even in the cartilage. Of the 6 eels digested with artificial gastric juice, all were found to contain metacercariae in their muscle tissues. The average number of metacercariae recovered from the 6 eels was 1219, with a range of 50 to 3762. These metacercariae, when fed orally to immunodeficient (scid) mice, developed into adult worms which were identified as *Procerovum cheni* Hsu 1950. The naturally infected eels were transferred to a new pond without snails and their eye lesions were not apparent anymore after 2 wk. In a follow-up investigation, 19 of 20 apparently healthy eels in a nearby aquaculture farm were found to harbour metacercariae in their muscles. However, the number of the metacercariae ranged from 1 to 14, with an average of 4.21. This is the first report of heterophyid metacercariae causing mass morbidity in aquacultured eels.

KEY WORDS: Eel · Heterophyid metacercariae · Eye lesion · Taiwan *Procerovum cheni*

INTRODUCTION

Although infection in freshwater fishes by cercariae of many digenetic trematodes usually results in the formation of metacercariae in the fish musculature without clinical signs, cercariae of *Diplostomum* spp. and *Tylodelphys clavata* are known to penetrate into the eyes causing cataracts (Wootten 1974, Hubert & Justine 1990, McCloughlin & Irwin 1991) and those of *Uvulifer ambloplitis* into the skin to form 'black spots' (Post 1987). Thus, cercariae can penetrate into the fish not only through the skin but also through the cornea of the eyes. Superinfection by helminths or protozoa in a closed aquaculture system is a common phenomenon due to the high density of fish in each pond. This might have contributed to the mass mortality of the farmed eels as illustrated by an outbreak of anguillicolosis in Taiwan (Ooi et al. 1996).

In this paper, we present an account of the outbreak of heterophyidiosis with eye lesions in an eel farm in Yunlin county, central Taiwan, and the identification of the trematode.

MATERIALS AND METHODS

Study site. In late July 1996, 90% (25 000 of 28 000) of 15 mo old aquacultured Japanese eel *Anguilla japonica* in 2 ponds of an aquaculture farm in Yunlin county, central Taiwan, were observed to have swollen, cloudy and white eyes. There was no mortality among the affected eels. Six morbid eels were brought to our laboratory for examination.

Parasitological, pathological and bacteriological examination. The 6 affected eels were killed under cold immobilization and the tissue around their eyes macerated on a slide glass for microscopic examination. Their skeletal muscles were then minced in a blender followed by digestion in 1% pepsin solution at pH less

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than 2 adjusted by adding concentrated hydrochloric acid. The digestion was carried out for 4 h using a magnetic stirrer in an incubator set at 37°C. After sedimentation and washing with saline of the digestant, the sediment was placed in a calibrated petri dish and then observed under a dissection microscope. Metacercariae that were obtained were then studied under a light microscope or administered orally to experimental mice or chickens.

The digestive tract, swimbladder, kidney, liver, heart, gill, eye, cartilage and muscle of the eels were fixed in Bouin's solution, dehydrated in an alcohol series, cleared in xylene, embedded in paraffin and sectioned at 4 µm. The sections were then stained with Haematoxylin-eosin and observed under a light microscope.

Samples of liver and kidney were taken and cultured in tryptone soya agar at 24°C for more than 48 h to check for bacterial infection.

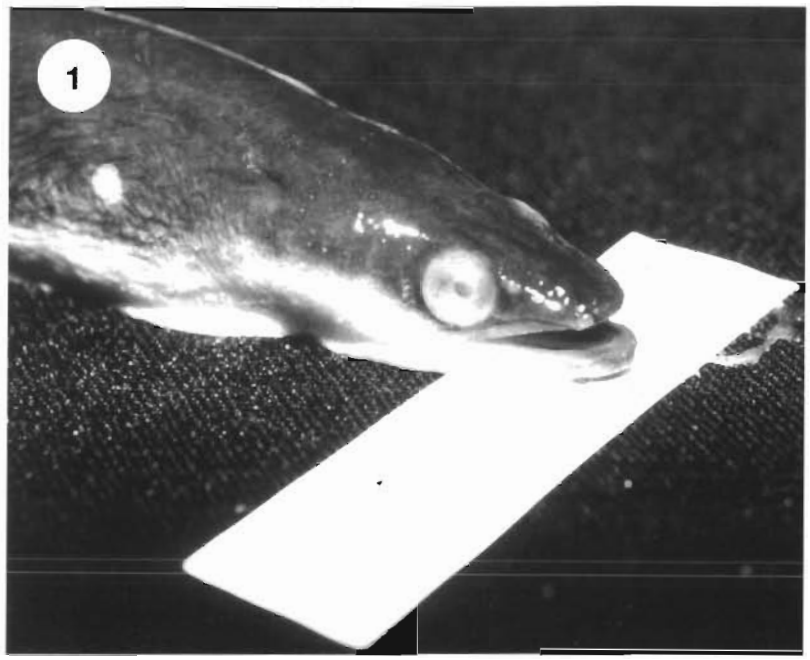

Fig. 1. *Anguilla japonica*. Affected Japanese eel with swollen, cloudy and white eyes

Experimental infection. To obtain the adult worms, metacercariae isolated from the digested muscle of the eels were orally fed to 4 immunodeficient (scid) mice and 2 chickens at a dose of 500 metacercariae ind.$^{-1}$. Two mice and the 2 chickens were killed 2 wk post-infection (PI), and the 2 remaining mice at 3 and 4 wk PI, respectively. Their digestive tracts were slit in warm saline and macerated with optical forceps; vigorous pipetting was then carried out to flush the minute flukes from the mucosa. The flukes were then collected under a dissection microscope.

The flukes were fixed in 70% alcohol, and this was followed by staining with alum-carmine solution for 1 h. The worms were then partially destained in 1% acetic acid in 70% alcohol under constant agitation for several minutes. The stained worms were then dehydrated in an alcohol series, cleared in xylene and mounted on a glass slide in Entellan (Merck Co.).

Follow-up study. In a follow-up investigation in April 1997, 20 apparently healthy eels in a nearby aquaculture farm were also examined for metacercariae in their muscle by digesting with artificial gastric juice solution as described above. The number of metacercariae collected were counted for each eel.

RESULTS

Gross examination of the affected eel showed swollen, cloudy and white eyes (Fig. 1). Numerous heterophyid metacercariae were observed in the muscles tissues around the eyeballs of all of the 6 eels examined. Metacercariae were collected from the digested muscle of all the eels (Fig. 2). The average number of metacercariae recovered from the 6 eels was 1219, with a range of 50 to 3762. No bacterial growth was observed in culture plates inoculated with the liver and kidney samples of the eels.

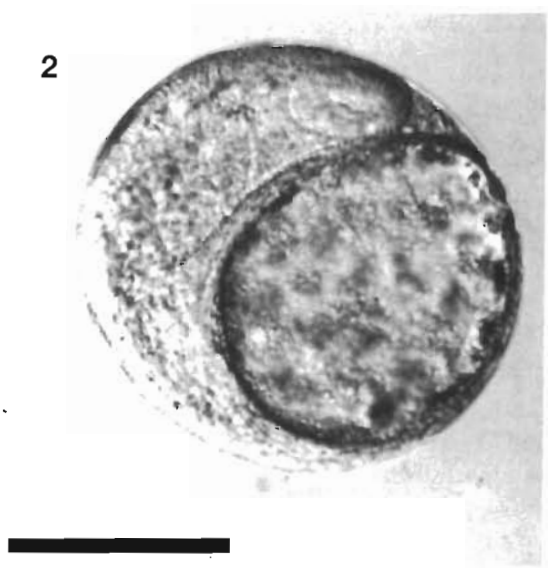

Fig. 2. *Procerovum cheni*. Metacercaria from digested eel muscle. Scale bar = 0.1 mm

Fig. 3. *Procerovum cheni*. Adult worm recovered from a mouse at 14 d after experimental infection. Alum-carmine stained. Scale bar = 0.1 mm

Adult worms were obtained from the small intestine of all the experimentally infected mice but none from the chickens. The numbers of worms recovered from the 2 mice on Day 14 PI were 6 and 11, and those recovered from single mice at Days 21 and 28 PI were 19 and 22, respectively. The adult worms were identified as *Procerovum cheni* Hsu 1950. Identification of the trematode was based on the description by Hsu (1950) and Pearson & Ow-Yang (1982). Briefly, the trematode has a single testis, and numerous minute spines in its gonotyl; both ceca extend to the upper level of the testis (Fig. 3). *P. cheni* is characterized by the fact that its metacercaria has an extraordinarily large excretory bladder.

Histopathological examination of the affected eels showed lesion of the lens and edema of the eye ball (Fig. 4). Hemorrhage of the tissue around the eye was also observed (Fig. 5). However, no inflammatory reaction of the host towards the encysted metacercariae in the muscle could be seen (Fig. 6). Metacercariae were also observed in the subcutaneous tissue below the skin (Fig. 7) and in the cartilage (Fig. 8).

Since we suspected that the eye lesion might be caused by the penetration of cercariae from the pond water, we suggested that the affected eels be transferred to a new pond without snails. Tens of thousands of snails could be seen at the bottom of the original pond after the water was drained (Fig. 9). Most of the snails were identified as *Melanoides tuberculata* Müller 1774 (Mollusca: Thiaridae), which is the synonym for *M. formosensis* Smith 1878 and *M. chinensis* Neville 1884 (Habe 1978). However, no attempt was made to identify the cercariae in these snails. Two weeks after the affected eels were transferred to a new pond without snails, their eye lesions were no longer obvious.

In the follow-up investigation, 95% (19 of 20) of the apparently healthy eels were found to harbour metacercariae in their muscles. However, the number of the

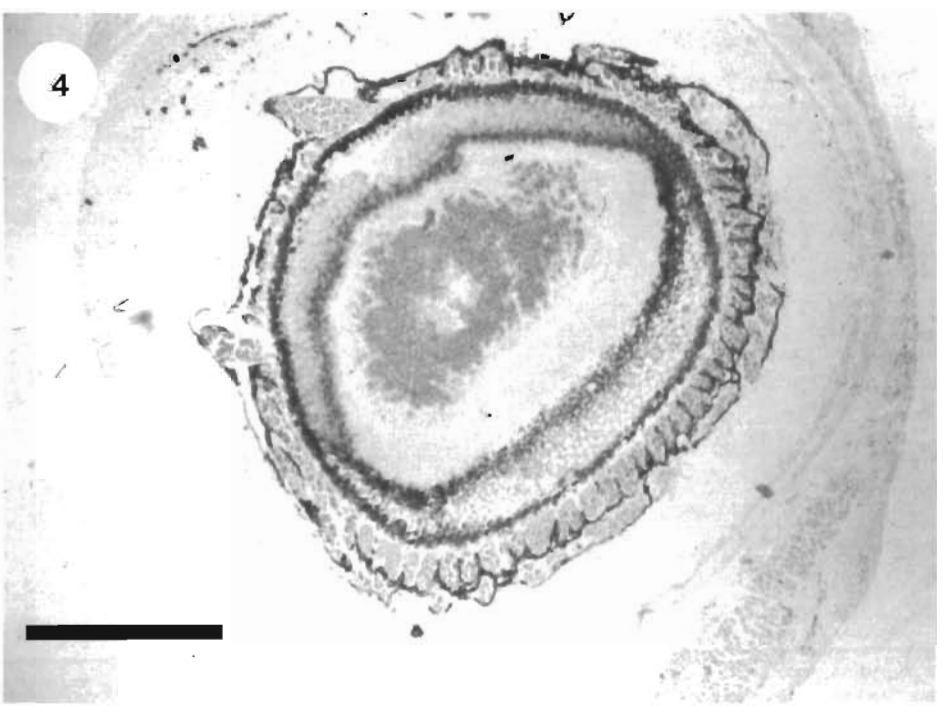

Fig. 4. *Anguilla japonica*. Histological section of the eye of Japanese eel showing lesion of the lens and edema around it. H&E stained. Scale bar = 0.5 mm

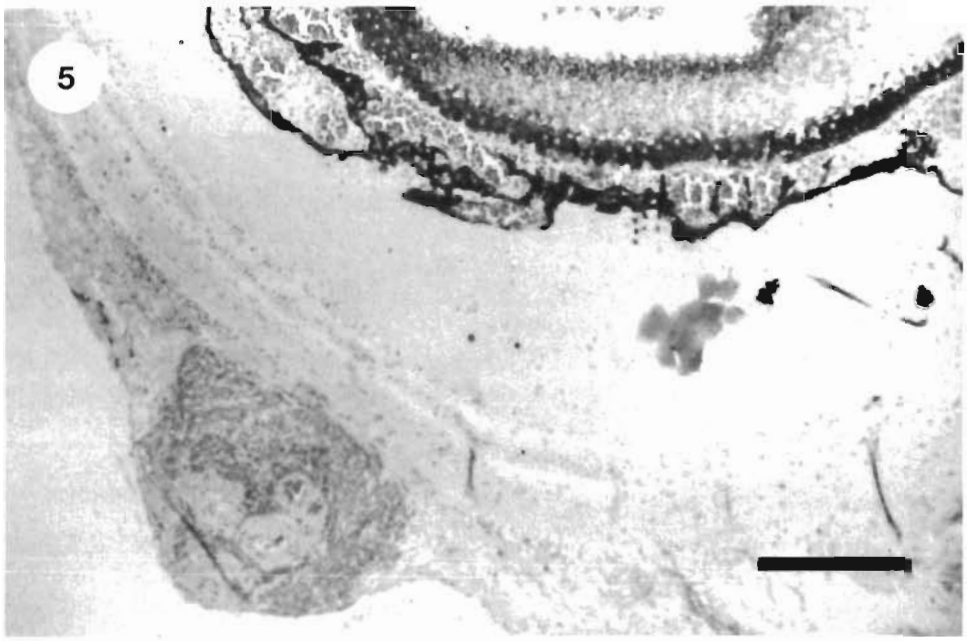

Fig. 5. *Anguilla japonica*. Histological section of the eye of Japanese eel showing hemorrhage adjacent to the eye capsule. H&E stained. Scale bar = 0.2 mm

metacercariae ranged from 1 to 14, with an average of 4.21. Of the 80 metacercariae collected from the 19 eels, 53 (66%) were identified as those belonging to *Procerovum cheni*. The remaining 27 metacercariae could not be identified due to the fact that they were still undeveloped, immature or had already degenerated.

DISCUSSION

This is the first paper that gives an account of mass morbidity of Japanese eels attributed to heterophyid metacercariae infection in an aquaculture farm. We cannot rule out the possibility that there may be other causes for the eye lesion besides heterophyidiosis, such as deficiency of dietary zinc (Ketola 1979). However, since transferring the affected eels to a new pond without snails led to a quick recovery of the eels' eyes, it is rational to say that the cause of irritation to the eels' eyes had been removed, resulting in quick recovery. We postulated that the eye lesions might have been caused by the irritation produced by the penetration of cercariae into the cornea of those eels, as evidenced by the damage to the lens and hemorrhage of

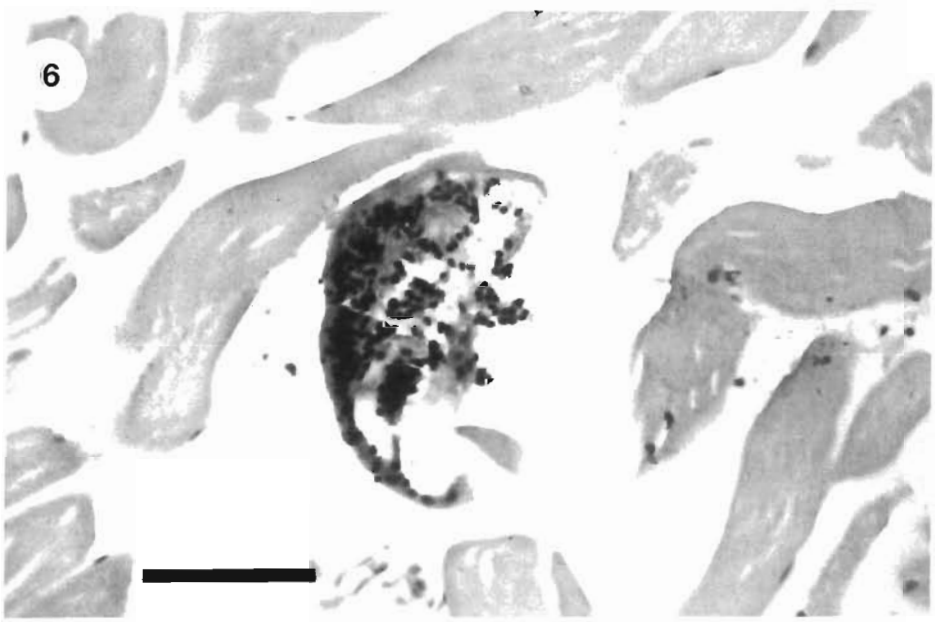

Fig. 6. *Anguilla japonica*. Histological section of the muscle of Japanese eel showing no inflammatory reaction to the metacercaria. H&E stained. Scale bar = 0.05 mm

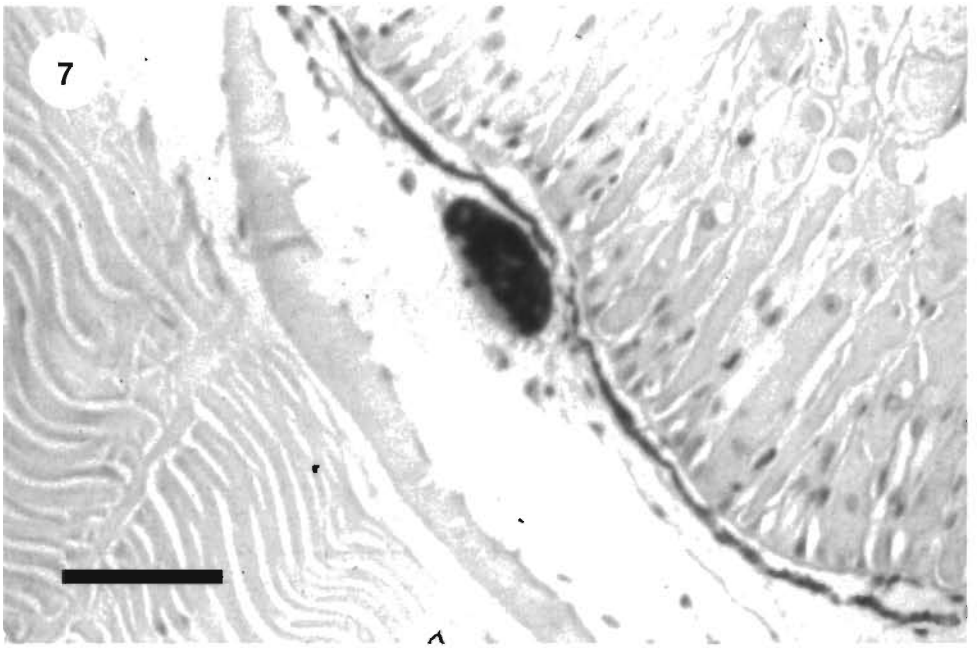

Fig. 7. *Anguilla japonica*. Histological section of the skin of Japanese eel showing no inflammatory reaction to the metacercaria under the skin. H&E stained. Scale bar = 0.05 mm

the tissues around the eyes. As a host response to this irritation, the eyes of the eels may have produced secretion.

Procerovum cheni belongs to the *Haplorchis* group of trematodes whose members are parasites of fish-eating birds and mammals, including man. Haplorchid metacercariae are abundant in many freshwater fishes in Taiwan (Ooi & Chen 1997, Ooi et al. 1997). The first and second intermediate hosts for *P. cheni* have been reported to be *Melanoides tuberculata* and *Macropodus opercularis*, respectively (Hsu 1950, Pearson & Ow-Yang 1982). Our finding of abundant *M. tuberculata* in the eel pond supports our postulation that mass release of cercariae from these snails might have occurred, resulting in the mass infection of the eels. Fortunately, since eel is not eaten raw, the probability of parasitic zoonosis occurring is low. However, we cannot absolutely rule out such a possibility.

Metacercariae of *Diplostomum* spp. and *Tylodelphys clavata* are known to localize in the eyes, including localization within the lens and retina of fishes, resulting in the formation of cataracts (Wootten 1974, Hubert & Justine 1990, McCloughlin & Irwin 1991). Our finding of eye lesions in the eels may resemble such a

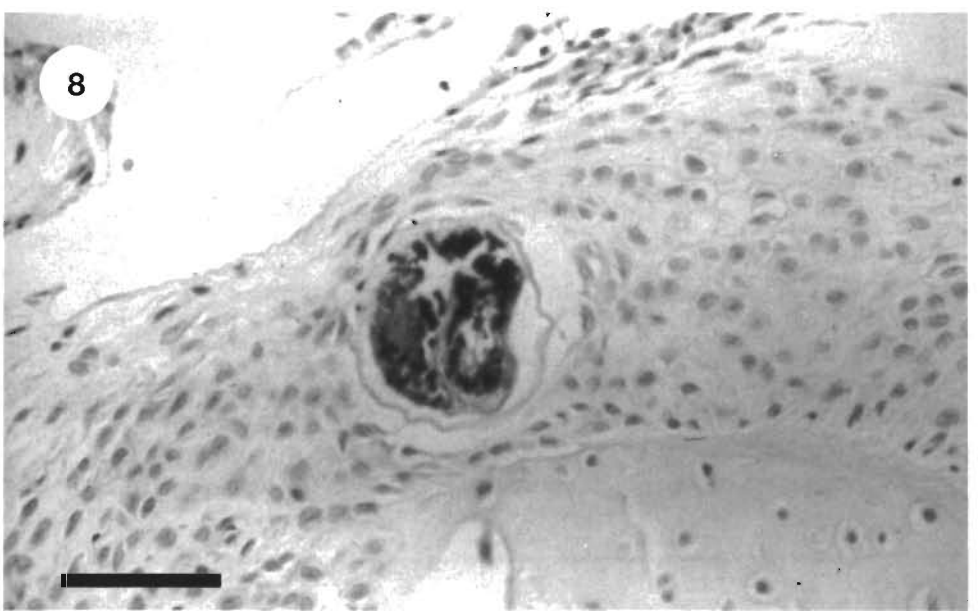

Fig. 8. *Anguilla japonica*. Histological section of the cartilage of Japanese eel showing a metacercaria within the cartilaginous tissue. H&E stained. Scale bar = 0.05 mm

Fig. 9. General view of the bottom of the pond which was used to culture the affected eels. The water had been drained and the presence of numerous snails could be seen

phenomenon but it might be associated with the penetration of the cercariae of *Procerovum cheni*. The fact that we observed metacercariae in the subcutaneous and cartilaginous tissues of the eels suggests that the cercarial penetration capability of this trematode species might be more than sufficient for it to penetrate through the skin instead of only through the eyes.

Since Taiwan is situated in a subtropical region, the water temperature in an open-air aquaculture pond may be more than 25°C during the summer months. Such a high water temperature might have contributed to the high levels of activity of the cercariae in the pond water (Post 1987). This, together with the high density of the eels in the culture pond, might have led to the high prevalence and intensity of the metacercariae in the eels.

Thus, control of heterophyid metacercaria infection will depend upon control of the first intermediate snail host and the definitive fish-eating avian host in and around the cultured eel ponds.

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Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: July 7, 1998; Accepted: September 12, 1998
Proofs received from author(s): December 14, 1998