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Incidence and histopathology of encysted progenetic metacercaria of *Clinostomum complanatum* (Digenea: Clinostomidae) in *Channa punctatus* and its development in experimental host

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ABSTRACT

Objective: To study the incidence of encysted progenetic metacercariae of *Clinostomum complanatum* (*C. complanatum*) in *Channa punctatus* (*C. punctatus*), associated histopathology and the experimental infection to laboratory chicken to obtain ovigerous adult worms. **Methods:** Live *C. punctatus* were brought from local fish market of Aligarh, India, dissected and examined on a monthly basis for the presence of *C. complanatum* cysts. For histochemistry, infected tissue sections with attached cysts were processed for haematoxylene and eosin staining. Cysts were aseptically fed to 4 day old leghorn chicken to obtain adult worms. Mechanically excysted metacercaria and the ovigerous adult worms were stained in carmine to prepare permanent slides. **Results:** One year survey for the infection of encysted progenetic metacercaria of *C. complanatum* in *C. punctatus* revealed the prevalence, intensity and abundance of 24.7%, 2.27 and 0.608, respectively. Histopathology showed heavy infiltration of immune cells at the site of cyst attachment and some tissue damage was also evident. Following feeding to experimental chicken, about 41.07% of the encysted metacercariae were able to excyst and migrate back to bucco-pharyngeal region where they tenaciously attached and fed on blood, and transformed into ovigerous adult worms from 62 hours onwards of post infection. **Conclusions:** The parasite is potentially pathogenic to the host, and the availability of a suitable intermediate host can be a contributing factor for the occurrence of *C. complanatum* metacercaria either in the excysted or encysted form, indicating loose host specificity and zoonotic potential.

1. Introduction

Fishes are good source of quality protein, but various diseases including parasitic infections pose a threat to fish culture[1]. In addition to the economic loss to farmers, many of the parasites, particularly trematodes, are also of zoonotic importance. Eating raw or, improperly cooked or processed fish is the main source of these infections to human that has been reported from various geographical regions[2], causing laryngitis. The World Health Organization (WHO) has estimated that the number of people currently infected with fish-borne trematodes exceeds 18 million, and many more are at risk[3].

Clinostomum complanatum (*C. complanatum*) (Rudolphi, 1814), a common clinostomid trematode found in the esophagus of ardeiid birds is widely distributed across the world, and in India the infective larval stage of this parasite is usually found in the body cavity of forage fish, *Trichogaster fasciatus* (*T. fasciatus*) (syn: *Colisa fasciata*), as non-encysted metacercariae with an infection rate of 98%-100%[4]. However, during our recent survey, it was observed that the encysted form of the progenetic metacercariae of this species was also found in the economically important food fish, *Channa punctatus* (*C. punctatus*) (Bloch, 1793), which serves as the second intermediate host for this parasite. The metacercariae of *C. complanatum* were known to cause considerable damage to the viscera and musculature of many fish species[5], however, the damage exerted to *C. punctatus* is not known.

Abidi and Nizami[6] successfully established the

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experimental infection of non-encysted metacercaria of *C. complanatum* from *T. fasciatus* in chicken, but there is no report of such studies on the encysted form of this parasite from *C. punctatus*. In the present study, a survey was carried out for one year to assess the incidence of infection in *C. punctatus* and the encysted progenetic metacercariae of *C. complanatum* obtained from the infected fish were fed to experimental leghorn chicken and their maturation into ovigerous adult stage was studied.

2. Materials and methods

2.1. Epidemiological study

Live *C. punctatus* of 7-16 cm long size were brought in pond water from local fish market of Aligarh (latitude: 27°, 54′ N; longitude: 78°, 05′E), U.P., India, to the lab on a monthly basis and maintained in aquaria until used. Within three days, these fishes were observed externally for the presence of metacercarial cysts, commonly known as yellow grub. The dissected fishes were carefully observed for the presence of encysted progenetic metacercariae of *C. complanatum* in all the internal organs. The attached cysts visible to the naked eye were photographed and then removed and transferred into 0.75% normal saline and their number was recorded systematically. The cyst wall was teared carefully using forceps to liberate the metacercaria which was fixed in 70% alcohol and permanent slides were prepared after staining in acid carmine.

2.2. Histopathological study

Some cysts with attached tissue of the infected fish were immediately washed and fixed in 10% neutral buffered formalin, processed further and embedded in paraffin wax to cut 7 μm thick sections using a rotatory microtome, stained with haematoxylin and eosin to study histology and cellular infiltration at the site of attachment. The DPX mounted sections were observed under microscope and photographed.

2.3. Experimental infection to chicken

Properly washed 4 cysts were carefully removed from the infected fishes and fed to each of the 14 four-day-old leghorn chicken using a soft brush, which were maintained in separate cages and provided commercially available chicken food and water *ad libitum*. The buccal cavity of each chick was regularly observed to detect the appearance and attachment of the parasite and they were necropsied daily until seven days post infection (p.i.). Developmental stages and adult worms obtained from chicken were stained in acid carmine to prepare permanent slides. The voucher specimens were submitted in the Museum of the Zoology Department, A.M.U. Aligarh.

3. Results

3.1. Epidemiology

The body surface of the fish *C. punctatus* appeared normal and no cyst was found to be attached on it. Following necropsy, the infection was largely found at the dorso-lateral sides of pre-opecular region either as an isolated or clumps of cysts (Figure 1). These cysts were visible with naked eyes (2-3 mm) and appeared yellow, light yellow or white colour. In addition, three cysts on the gill and eight on the kidneys were also found attached in different fishes. From the microscopic studies of mechanically excysted metacercaria, it is reported for the first time that the progenetic metacercaria is *C. complanatum*, which was found in the encysted form in the intermediate host, *C. punctatus* (Figure 2), unlike the excysted form occurring in *T. fasciatus*. Interestingly, the size of the host fish had no correlation with the infection rate, but the size of the worms was comparatively bigger than that of non-encysted metacercaria and showed active movement once they were mechanically excysted. The morphological features corresponded well with that of non-encysted metacercaria from *T. fasciatus*, which were characterized by the presence of bifurcated gut extending to posterior most region of the worm, quiescent gonads, and narrow streak like uterine tube extending posterior to acetabulum and the position of gonads, *etc*.

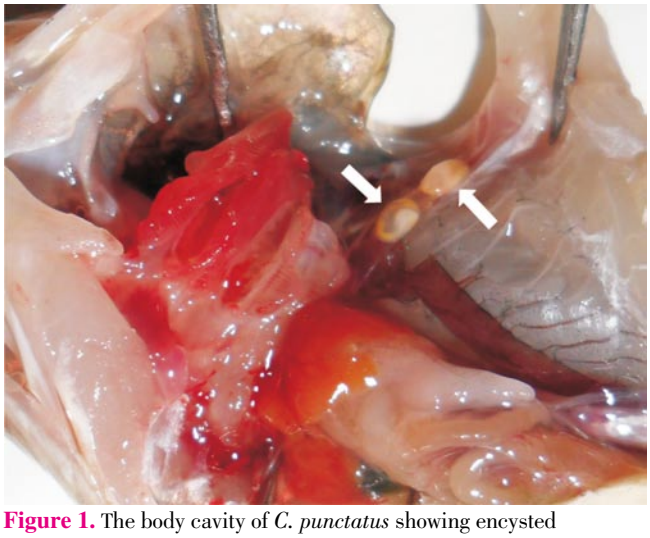

Figure 1. The body cavity of *C. punctatus* showing encysted progenetic metacercaria of *C. complanatum* (arrows).

In the present study, the prevalence of encysted progenetic metacercariae of *C. complanatum* infection in *C. punctatus* was quite varied from month to month. It was maximum in December (52.6%), followed by January (48.1%), while infection was not detected (0%) during October and minimum infection was observed in September (7.6%). The range of cysts varied between 1 and 12 per infected fish. The mean intensity was maximum in July, whereas the highest