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Transplantation of *Cardicola opisthorchis* (Trematoda: Aporocotylidae) sporocysts into the intermediate host, *Terebella* sp. (Polychaeta: Terebellidae)

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ABSTRACT

Cardicola opisthorchis is a blood fluke pathogen significantly affecting cultured Pacific bluefin tuna *Thunnus orientalis* in Japan. It is known that the intermediate host of *C. opisthorchis* is a terebellid polychaete *Terebella* sp. In order to study the intrapolychaete larval development of *C. opisthorchis*, we transplanted sporocysts, which contained a large number of cercariae, of *C. opisthorchis* obtained from *Terebella* sp. into sporocyst-free *Terebella* sp., which had been maintained at 20 °C. The transplanted sporocysts switched from cercarial to sporocystal production by 17 days after transplantation (d.a.t.) and daughter sporocysts were released into the polychaete body cavity at 25 d.a.t. Subsequently, the released daughter sporocysts produced daughter sporocysts again. Thereafter, daughter sporocysts that contained cercariae appeared at 38 d.a.t. and gradually increased. At 51 d.a.t., 136 sporocysts that had multiplied from the original two transplanted sporocysts were observed in the body of one polychaete, and cercariae were released from daughter sporocysts inside the polychaete body cavity. Subsequently the cercariae were found to be released outside the polychaete at 57 d.a.t. This is the first successful case of in situ observation of the development of a blood fluke within the intermediate host.

1. Introduction

Blood fluke infection with *Cardicola opisthorchis* is one of the main causes of mortality in cultured Pacific bluefin tuna *Thunnus orientalis* in Japan [1,2]. It is important to reveal the life history of pathogens to develop effective control measures. In our previous study, we elucidated that the intermediate host of *C. opisthorchis* was a terebellid polychaete *Terebella* sp., in which the sporocysts contained cercariae [3]. Subsequently, we found sporocysts that contained sporocysts, suggesting the possibility that the sporocysts multiplied within the polychaete hosts, namely sporocystogenous sporocysts [4]. There are few reports about larval development of aporocotylids inside intermediate hosts. In schistosomes, transplants of sporocysts to the intermediate host have been conducted in *Schistosoma mansoni*, *S. haematobium*, *S. bovis* and *S. japonicum* [5–8]. Furthermore it was found that the daughter sporocysts of *S. mansoni*, which were produced through sporocystogenesis by sporocysts, were capable of stopping production of cercariae and orienting production towards an additional generation of sporocysts [9]. In order to study the intrapolychaete larval development of *C. opisthorchis*, we tried to transplant the sporocysts of *C. opisthorchis* that contained a large number of cercariae into *Terebella* sp. In this paper, we report the results of the transplantation experiment of sporocysts and discuss the multiplication and development of the larval stages of *C. opisthorchis*.

2. Materials and methods

2.1. Donor polychaete

We selected a terebellid polychaete *Terebella* sp. that contained a lot of sporocysts of *C. opisthorchis* out of the polychaetes obtained from ropes attached to tuna culture cages at the coast of Tsushima Island, Nagasaki, Japan on November 25, 2014. The sporocysts contained a large number of cercariae.

2.2. Recipient polychaetes

Terebella sp. was collected from the ropes attached to tuna culture cages at the coast of Tsushima Island on October 28, 2014. These polychaetes were flattened between a slide glass and a coverslip, and were checked for having no sporocysts through their body wall with a microscope. They were maintained in sterile seawater for approximately one month before transplantation to confirm that there was no oversight of the sporocysts or infection of miracidia.

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2.3. Transplantation

The donor polychaete was dissected under a stereoscopic microscope, and the sporocysts were sucked up with a 1-ml syringe equipped with a 26G needle and injected into the body cavities of five recipient polychaetes, three to five sporocysts into each polychaete. These polychaetes were individually put in 25-cm^2 cell culture flasks (Iwaki) with sterilized seawater supplemented with 1% Antibiotic-Antimycotic (100×) (Gibco) and kept at 20 °C. They were fed with *Pavlova lutheri* and the breeding water was changed twice a week. The conditions of internalized sporocysts within the polychaetes were observed under an optical microscope every 3–10 days by flattening these polychaetes between a slide glass and a coverslip.

3. Results

Among the five injected polychaetes, we succeeded in transplanting four sporocysts that contained a large number of cercariae into the body cavities of two polychaetes (Fig. 1A), but failed to transplant into the remaining three polychaetes because either the sporocysts died or leaked from the body cavities of polychaetes. In both the polychaetes with two injected sporocysts each, daughter sporocysts were seen inside the transplanted sporocysts together with cercariae 17 days after transplantation (d.a.t.) (Fig. 1B), and three daughter sporocysts were observed in the body cavity of one polychaete at 25 d.a.t. (Fig. 1C and 2A). The transplanted sporocysts were still filled with daughter sporocysts even after having released the initial daughter sporocysts. As the released daughter sporocysts grew, daughter sporocysts of the next generation were seen in their bodies (Fig. 1D). The sporocysts increased in number in the polychaetes from injected sporocysts by switching from cercarial to sporocystal production and repeatedly producing daughter sporocysts (Fig. 3).

Daughter sporocysts that contained cercariae began to be seen at 38 d.a.t. (Fig. 1E), and the ratio of this type in the total number of sporocysts increased to more than 30% at 46 d.a.t. In one polychaete sporocysts multiplied to 136 from the initially transplanted two sporocysts at 51 d.a.t. (Fig. 2B and 3). On the same day cercariae released from sporocysts were seen inside the polychaete body (Fig. 1F). However, the other polychaete died at 51 d.a.t. (Fig. 3).

As for the one polychaete that survived, 14 cercariae were released outside the polychaete body at 57 d.a.t., and cercarial accumulation was observed inside the bulges of the ventral base of the notopodia of the polychaete body cavity (Fig. 2C). On the next day, a release of 105 cercariae was seen, but the polychaete died on the following day.

The length of sporocysts transplanted into polychaetes were approximately 450–820 (675) μm (n = 4), but these sporocysts grew afterwards and their length reached approximately 1120–1330 (1200) μm (n = 4) at 38 d.a.t. On the other hand, the length of subsequent daughter sporocysts, both sporocystogenous sporocysts and cercariogenous sporocysts, ranged from approximately 120 to 900 μm. In addition, the transplanted sporocysts looked more blackish (darker in color) than the daughter sporocysts under the microscope. Therefore, the transplanted sporocysts could be easily distinguished from daughter sporocysts by their size and darker more blackish body color (Fig. 2D).

4. Discussion

In this study we succeeded in transplanting sporocysts into the body cavity of polychaetes, because the transplanted sporocysts, that contained a large number of cercariae, produced daughter sporocysts. That is, the cercariogenous sporocysts that we transplanted were shown to change into sporocystogenous sporocysts within the polychaete body cavity. Moreover, because the transplanted sporocysts were filled with daughter sporocysts even after they released daughter sporocysts, it is probable that the daughter sporocysts were repeatedly produced and released. In addition, because the released daughter sporocysts produced daughter sporocysts, it is strongly suggested that

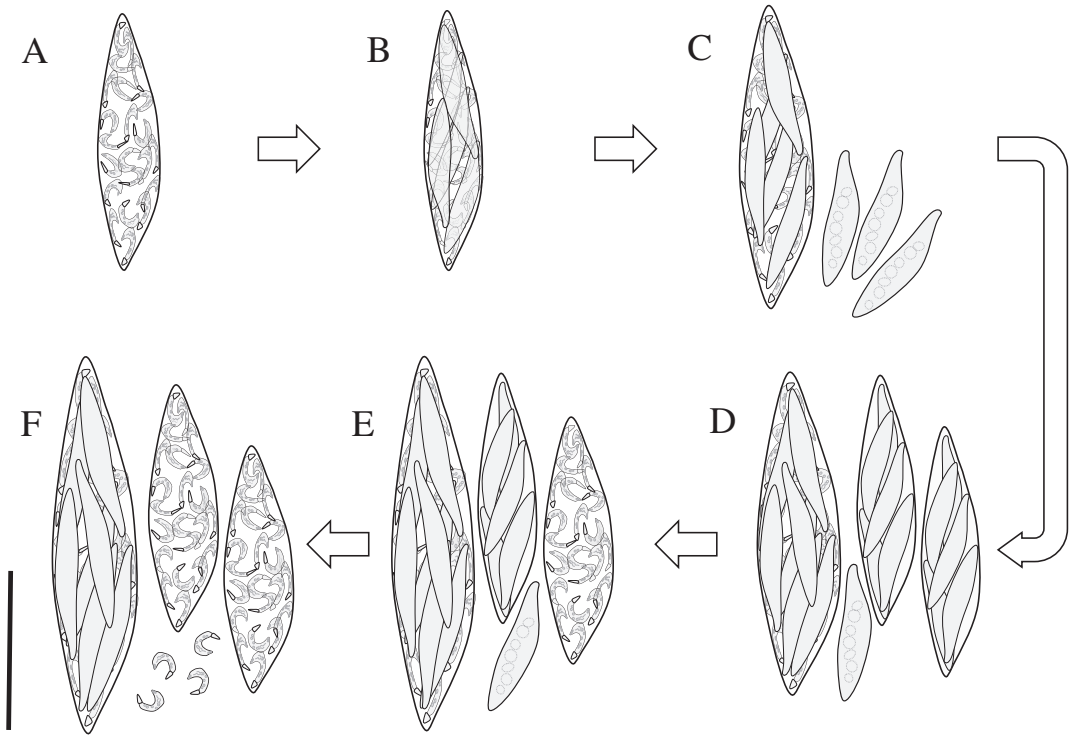

Fig. 1. Morphological changes of sporocysts of *Cardicola opisthorchis* inside the recipient polychaete, *Terebella* sp. (A) Transplanted sporocyst; sporocyst containing a large number of cercariae. (B) At 17 d.a.t.; daughter sporocysts were seen inside the sporocyst. (C) At 25 d.a.t.; three daughter sporocysts were released from the first generation sporocyst. (D) At 31 d.a.t.; further daughter sporocysts were seen inside the released daughter sporocysts. (E) At 38 d.a.t.; daughter sporocysts that contained cercariae were seen. (F) At 51 d.a.t.; free swimming cercariae were seen inside the polychaete. Scale bar 500 μm.