The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections

Received 20 September 1994 / Accepted: 30 January 1995

**Abstract** Hatched, ensheathed third-stage larvae of *Contracaecum osculatum*, 300–320 μm long, were shown to be infective to copepods, to nauplius larvae of *Balanus* and to small specimens of fishes (sticklebacks, 0-group eelpout). Other fishes such as gobies and small flatfishes became infected by ingesting infected crustaceans. Cod were infected by being given infected small fishes. In the crustacean paratenic hosts, little growth of the larvae occurred, if any. In the liver sinusoids of sticklebacks and gobies the length of most of the unencapsulated third-stage larvae had not even doubled within 6 months of infection. The fate of larvae (max. 2 mm long) given to cod via infected intermediate fish hosts was apparently decided by the size of the larvae only. Small larvae became encapsulated and eventually died in the liver and wall of the gastrointestinal tract. Larger larvae migrated to the liver parenchyma, where some grew to a length of as much as 10 mm. The growth of the larvae in sticklebacks was shown not to be affected by an increase in temperature (infected fish being transferred from 8° to 14° and 20° C), by the intensity of infection and, partly, by the age of infection (e.g. some 2-week-old and 6-month-old larvae were of identical size). In the liver and mesentery of plaice the third-stage larvae developed via copepod paratenic hosts to infectivity (i.e. to more than 4 mm in length), showing that the life cycle may be completed with an optional paratenic invertebrate host and only one intermediate fish host. In combination with earlier results showing that the ensheathed third-stage larva (not the second stage) emerges from the egg and with literature data on the occurrence of larvae in fishes and the presence of fourth-stage larvae and adults predominantly in the stomach of grey seals, the life cycle of *C. osculatum* is shown experimentally for the first time.

**Introduction**

The adults and fourth-stage larvae of nematode species of the genus *Contracaecum* Raillet & Henry, 1912 (Ascaridoidea, Anisakidae) are found in the digestive tract of seals, some dolphins and piscivorous birds; their third-stage larvae are known to occur in fishes, usually encapsulated in the viscera. Although the life cycles of some avian species of *Contracaecum* are known, those of the mammalian species have not yet been elucidated experimentally. Especially, the way the larvae are transferred to the fish hosts has not been determined for these species.

The main final host of *C. osculatum* (Rudolphi, 1802) in the Baltic Sea is the grey seal. Electrophoretic isozyme studies have indicated that in the Atlantic Arctic-Boreal region, *C. osculatum* sensu lato includes at least three sibling species that are reproductively isolated and genetically differentiated, with differences in their geographical distribution and host preferences being reported (Nascetti et al. 1993), although the sibling species have thus far not been found to be morphologically distinct (cf. Fagerholm 1988). Nascetti et al. (1993) concluded that the name *C. osculatum* should be used only for species C, redescribed from the grey seal from the Baltic Sea (Fagerholm 1989).

In the present study, attempts were made to transmit *C. osculatum* sensu stricto experimentally to crustacean and fish hosts to determine the role of various potential hosts in the life cycle of this seal parasite and to relate these results to observations on larvae occurring in fish catches. Micrographs of the eggs and the first-, second- and ensheathed third-stage larvae from crushed and naturally hatched eggs have been shown in an earlier paper (Køie and Fagerholm 1993).
Eggs were obtained from specimens of *Contracaecum osculatum* removed from the stomach of three specimens of grey seal, *Halichoerus grypus*, from the northern Baltic Sea (0.6% salinity) off the Swedish coast on 11 November 1992 and off the Åland Islands on 24 August 1993. The seals had been accidentally drown in fishing gear. Most eggs were incubated at 6°C in seawater (3% salinity). To accelerate development, some eggs were kept at about 15°C. The ensheathed third-stage larvae from hatched eggs were kept in seawater at 6°C. Laboratory-reared *Acartia tonsa* (Copepoda), nauplius larvae of *Balanus* sp. (Cirripedia) and various species of copepods caught in the Oresund were exposed to ensheathed larvae in 250-ml Pyrex bluecap bottles. The copepods and nauplius larvae of *Balanus* were kept at 12°C, fed on *Rhodomonas* sp. and treated as described by Køie (1991). Laboratory-reared harpacticoid copepods, amphipods (*Gammarus* spp., *Corophium bonelli*, *C. volutator*), isopods (*Idotea* sp., *Jaera albinons*, *Sphaeroma rugicauda*), mysids (*Neomysis integer*, *Praunus flexuosus*, *P. inermis*) and decapods (2–3 cm long: *Crangon cran- gon*, *Palaeon adspersus*) were repeatedly exposed to several hundred ensheathed larvae in a small amount of seawater (8°C–10°C). They were fed frozen food only.

To study whether larvae might be transmitted from one crustacean to another, *A. tonsa* harbouring at most 1-week-old larvae were fed to the above-mentioned malacostracans. Sticklebacks, *Gasterosteus aculeatus* and *Pangius pungitius*, and common goby, *Pomatoschistus microps*, and common goby, *Pomatoschistus microps*, were isolated in aquaria for one year; 0-group (6–10 cm long) plaice, *Pleuronectes platessa*, and 0-group laboratory-reared eelpout, *Zoarces viviparus* (3–5 and 10–12 cm long, respectively), were used as experimental fish hosts. All fishes were fed frozen food only. Some specimens of each species were examined as controls.

To study whether ensheathed third-stage larvae would be directly infective to fishes, four specimens of each species of the fish species nine- and three-spined sticklebacks, *Gasterosteus aculeatus* and *Pangius pungitius*, and common goby, *Pomatoschistus microps*, isolated in aquaria for one year; 0-group (6–10 cm long) plaice, *Pleuronectes platessa*, and 0-group laboratory-reared eelpout, *Zoarces viviparus* (3–5 and 10–12 cm long, respectively), were used as experimental fish hosts. All fishes were fed frozen food only. Some specimens of each species were examined as controls.

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To study the possible transmission of larvae from one fish to another, cod were given 0-group eelpouts and three-spined sticklebacks harbouring larvae of various ages. To study the influence of temperature on the development of the larvae in the fish intermediate host, four specimens of three-spined sticklebacks, all harbouring about 4-month-old larvae, were transferred from 8°C to aquaria maintained at 14°C and 20°C (3% salinity), respectively, for 2 months and compared with infected fishes kept constantly at 8°C.

Pieces of liver of sticklebacks and encapsulated larvae from plaice were fixed in acetic-alcohol-formalin (AAF), embedded in paraffin or Epon and stained with Heidenhain’s azan or toluidine blue. Larvae of various ages and sizes were fixed in Berland’s lactic acid and mounted in glycerol-jelly. Measurements are based on fixed specimens.

**Results**

Development of the third-stage larvae in the crustacean hosts

Free-swimming, ensheathed third-stage larvae (Fig. 1) were ingested by *Acartia tonsa* and various harpacticoid copepods. The ensheathed larvae migrated from the copepod intestine into the haemocoel. More than ten larvae could be found in individual copepods (Fig. 2). Even when infected with only a single larva, *A. tonsa* did not survive for more than 2 weeks. Within 1 week, merely a slight increase in worm length had occurred (Table 1). No harpacticoid copepod examined at 4 weeks post-infection (p.i.) was found to be infected. *Acartia sp.*, *Centropages hamatus*, *Paracalanus parvus* and *Temora longicornis* from the Oresund became experimentally infected. Ruptured cuticles of second-stage larvae were found in the copepod gut lumen. In these cases the infection was followed for 1 week only, and during this period the larvae grew only slightly (to a length of 360 μm). In this study, copepods could be experimentally infected by ensheathed larvae kept at a low temperature (4°C) even at 3 months after hatching.

Nauplius larvae of *Balanus* sp. from the Oresund became infected, and more than ten larvae could be found in cypris larvae examined at 1 week p.i. (Fig. 3, Table 1).

Although ensheathed larvae were ingested by all local species of mysids and ensheathed larvae could be found in the intestinal tract for up to 12 h after initial exposure, apart from one specimen of the mysid *Neomysis integer*, no malacostracan became infected by ingesting ensheathed larvae. In the single *N. integer* examined at 3 weeks p.i., about 20 larvae were found; all larvae occurred in the legs (Fig. 5). At 3 weeks of age, larvae measured 410–530 μm in length (Fig. 4, Table 1). Live larvae were found in the intestinal tract of mysids after ingestion of *A. tonsa* harbouring maximally 3-day-old larvae. No larva was found in the haemocoel of these mysids or in any other malacostracan exposed to infected *A. tonsa* on examination at 1 week p.i. No control crustacean was found to be naturally infected with larvae of *Contracaecum osculatum*.

Exposure of fishes to ensheathed third-stage larvae

When examined at 2 weeks p.i., two specimens of eelpout and one specimen of nine-spined stickleback con-