POST-EMBRYONIC DEVELOPMENT OF WARDIUM CHAUNENSE BONDARENKO ET KONTRIMAVICHUS, 1977 (CESTODA: HYMENOLEPIDIDAE) IN THE INTERMEDIATE HOST

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Abstract. The development of W. chaunense cisticercoid in the intermediate host, the oligochaete Rhycoclitus coccineus Veidlovsky, 1875 (Tubificidae) is described. This is the first record of oligochaetes serving as intermediate hosts of cestodes of the genus Wardium Mayhow, 1925 sensu Spassky et Spasskaya, 1954. The cisticercoid of W. chaunense differs in its morphology from other cisticercoids of this cestode genus and belongs to the type of ramicercous according to the classification by Skryabin and Matevosyan (1945).

Cisticercoids possessing hooks identical with those of Wardium chaunense were found in 20 (5 %) of the 400 oligochaetes Rhycoclitus coccineus (Tubificidae) examined in August 1974. The hosts were collected in the shore region of a tundra lake in Chama lowland (North-West Chukotka) often visited by common snipe, Gallinago gallinago L. The cisticercoids had numerous processes on the base of tail, resembling thus the cisticercoids of Monorcholepis djuardini (Krubbe, 1869) found by Demshin (1971) in Henlea ircestis (Euchytraeidae) in Primorye Territory. They differed, however, in the number of hooks.

Eleven specimens of Wardium chaunense were recovered from a young G. gallinago dissected at the beginning of September. The “eggs” obtained from these cestodes were used for experimental infection of Rh. coccineus and their development to mature cisticercoids was followed. The methods used and the morphology of various developmental stages of the cisticercoids are described below.

METHODS

Four cestode specimens were used for the experimental infection of intermediate hosts. The parts of strobiola containing proglottids with uteri were removed and placed in water at the temperature of 10 °C for 12 hours. The released “eggs” were then transferred into Petri dishes containing water and the oligochaetes Rh. coccineus were placed there for 10 hours. The oligochaetes were previously examined under the microscope to exclude a spontaneous infection, then repeatedly washed with water and kept in jars with humid moss at the temperatures of 18–20 °C. Two experiments were set out: 1. “eggs” from one strobiola were used for the infection of 190 Rh. coccineus, 42 (22.1 %) of them were infected; 2. “eggs” from three strobiolae were used for the infection of 230 Rh. coccineus, 35 (18.7 %) of them were infected. The development of the larvae was observed at the intervals of 1–3 days starting from the penetration of the oncospheres into the body cavity of the tubificid oligochaetes up to full maturity of the cisticercoids. The larvae of all stages were recovered from the body of the intermediate host, placed in water and then measured, drawn and photographed.

The mature cisticercoids were studied either alive or in polyvinyl alcohol. Sexually mature cestodes were fixed in 70% alcohol and stained with haematoxylin after Caraschi. One strobiola was fixed in polyvinyl alcohol for a detailed study of cirrus armament.

RESULTS

Development of *W. chaunense* in the intermediate host. The authors who studied the post-embryonic development of Hymenolepididae (Skrjabin and Matevosyan 1945, Voge 1967, Neradová-Valkounová 1971, Szelenbaum 1973 and others) differentiated the following stages of development: mobile oncosphere, growing oncosphere, elongated larva (megalosphere), differentiating larva (metamere), scolexogenesis, invading larva and cysticercoid.

The post-embryonic development of *W. chaunense* corresponds to the general scheme, but there is a peculiarity in the formation of tail appendage. It should be noted that in our experiments the intensity of infection was very high, up to 45 larvae, which seems to have retarded their development. Only in the cases when there were no more than five larvae, the development was more or less even and was completed within 32—34 days. Therefore we have no possibility to show the exact periods of individual stages of larvogenesis.

**Mobile oncosphere.** The oncospheres released from the embryonal envelopes in the intestine of the oligochaete actively penetrate into the body cavity and reach the site of location, the middle third of the oligochaete body. In heavy infections, the larvae are usually located also in the middle part of the body. In our experiments, the oncospheres were present in the body cavity already 12 hours after the contact of the oligochaetes with the "eggs" had ceased.

**Growing oncosphere** (Fig. 1A; Plate I, Fig. 1). The oncosphere is at first globular or slightly oval and then gradually grows up to 0.053—0.082 mm. On the surface, there are globular cells with well visible nuclei. The embryonal hooks are usually located in pairs on one of its poles. At the end of this stage of development the oncosphere becomes more elongated and a small, eccentric globular cavity appears in its centre.

**Elongated larva** (Fig. 1B; Plate I, Fig. 2) grows intensively on poles. It is linguiform and measures 0.119—0.209×0.107—0.111 mm. The cavity, which was previously eccentric, is slightly shifted to the future tail end and inside it appears a septum consisting of two visible layers of cells. The size of the upper cavity is 0.066×0.057 mm, of the lower one 0.078×0.062 mm. The upper cavity is lined with large, scarce globular cells. The lower cavity is outlined with a very thin membrane, along which are solitary globular cells. The embryonal hooks are dispersed no more strictly in pairs behind the septum separating the cavities.

**Differentiating larva** (Fig. 1C—D; Plate I, Figs. 3—7). The body is divided into two isolated segments. The anterior one is larger, measuring 0.23×0.12 mm, globular or oval, and the cavity in its centre measures 0.10—0.11×0.09—0.10 mm. The posterior segment is smaller, globular, and measures 0.07—0.09 mm in diameter. The anterior segment later transforms into the cysticercoid and the posterior segment into the cercoper. The tail appendage starts to grow intensively after the differentiation. At the end of this developmental stage it is either of the same length or twice as long as the anterior segment (0.12—0.52 mm). The embryonal hooks are distributed along the whole length of tail. The cavity is situated in the distal portion of the tail. Also the anterior segment becomes elongated and linguiform or ovoid, measuring 0.15—0.37×0.11—0.20 mm. The cavity measures 0.10—0.23×0.06—0.14 mm; it is gradually filled with large cells among which are adipose-like granules.

At the end of this stage, there appear small protrusions of the tegument near the anterior border of the tail appendage. They are at first spherical, then elongated and transform into the tail appendage. Their number is variable, but not exceeding 24. Solitary globular cells are visible inside the appendages. The final formation of the appendages occurs only after the invagination of the scoles.
Scolexogenesis (Figs. 1E—G, 2 A; Plate I, Figs. 8—9; Plate II, Figs. 1—3). After the appendages have appeared, there starts the isolation of the scolex and neck from the future cyst, manifested in the formation of the strangulation at the border between them. The anterior segment is pyriform. At the same time the cyst is separated also from the tail appendages. The formation of rostellum, sheath and suckers occurs almost simultaneously. The hooks are formed in the base of rostellum, the blades appearing first. The neck is elongated and a sinus and solitary calcareous bodies are visible inside it.

The capsule, the tissue which lines it and the muscular fibres serving probably for the evagination of the scolex are formed at the posterior end of the anterior segment. At the end of this developmental stage, the development of individual part of larvae is practically completed and their measurements are as follows: Scolex 0.30—0.42 × 0.25—0.27 mm, suckers 0.086—0.094 × 0.07—0.08 mm, rostellum 0.078—0.107 × 0.07 mm, hooks 0.037—0.041 mm, neck 0.18—0.24 × 0.14—0.15 mm, capsule 0.33—0.37 × 0.22—0.27 mm; tail 0.67—0.82 mm; appendages in its base up to 0.16 mm long. The sinus is visible along the whole neck.

Invaginating larva is characterized by an increased mobility of the scolex and neck. The invagination of scolex occurs very rapidly, most rapidly within several minute. We have examined a live oligochaete with three larvae at the stage before invagination every two hours, but we were unable to see the very process of scolex invagination. Only the larvae before or after invagination could be observed.

Fig. 1. Development of Wardium chaunense. A — stage of growing oncosphere, B — elongated larva, C—D — differentiating larva, E—G — early scolexogenesis.
Cysticercoid (Figs. 2 B—D; Plate II, Figs. 4—8). The cysticercoid cannot be considered fully formed after the invagination is completed. The outlet of the cyst is not yet tightly closed, the slightest pressure on the larva results in the evagination of scolex from the cyst cavity and the scolex itself, as well as the neck, have not yet occupied their usual site inside the cyst. There occurs the “ripening” of the larva manifested by an intensive formation of the cyst envelopes, their thickening, increased formation of calcareous bodies and growth of appendages in the base of tail. The formed cysticercoid may be assigned to the type “ramicercus” (Skryabin and Matevosyan 1945, Bondarenko and Kontrimavichus 1976) differing only in that the appendages in the base of its cercomer are of the same length as the basic stem, whereas in the typical ramicercus the tail is much longer than the appendages in its base and the appendages are less numerous.

Fig. 2. Development of Wardium chaunense. A — completion of scolexogenesis, larva before invagination, B — formed cysticercoid (internal structure), C — hook, D — formed cysticercoid (general view).

The cyst is oval, measuring 0.49—0.57 × 0.32—0.36 mm. A tightly closed outlet is situated at the anterior end. The outer envelope consists of a homogeneous layer 0.001 to 0.002 mm thick, with processes measuring 0.004—0.006 mm in length and running off deep into the lower layer. In live cysticercoids the lower layer appears to consist of fibrous structures, among which numerous cellular elements are situated. Numerous granules are accumulated near the outlet of the cyst and partly near its posterior end at the place where the tail is situated. The layer is 0.008—0.020 mm thick in the centre of the cyst. The neck is 0.010—0.016 mm thick and surrounds the scolex; its tissues contain numerous calcareous bodies. The scolex measures 0.29—0.36 × 0.31—0.32 mm. The suckers have a well developed musculature measuring 0.11—0.12 × 0.09—0.12 mm. The rostellum measures 0.13—0.14 × 0.08—0.010 mm and possesses a depression on the
anterior end, in which lie the hooks. The rostellar sheath measures 0.29—0.36 × 0.14 mm. Ten hooks of aploparaksoid type measure 0.041—0.045 mm (blade 0.023—0.025 mm, base with root appendage 0.033—0.035 mm, handle 0.006 mm, width of hooks 0.023—0.025 mm).

DISCUSSION

The life-cycles of three species of the genus *Wardium* have been described in the literature. Jarecka (1960) obtained cysticercoids of the parasite of ducks, *W. aequabile* (Rud., 1810) after experimental infection of crustaceans, *Cyclocypris laevis* (Müll.). According to the classification by Skryabin and Matevosyan (1945), the cysticercoids of this species belong to the type of ceroecyst, very common in Hymenolepididae. Podesta and Holmes (1970) obtained a larva of *W. paraporale* Podesta et Holmes, 1970, a parasite of *Podiceps caspicus*, after experimental infection of the crustacean *Hyalella arctica* (Saussure, 1858). The cysticercoid of *W. paraporale* figured by these authors has no tail appendage (unfortunately, the figure is schematic and the cysticercoid seems to be illustrated without outer envelopes). The authors write: “cysticercoid with ceroecmter oval, smooth-walled”, but they probably regard as ceroecmter the envelopes of cysticercoid, which are losted in the definitive host (this term is also discussed by Jarecka 1972, 1975). Pike (1968) found the cysticercoids of *W. cirrosum*, a parasite of gulls, in leech, *Herpobdella octoculata* L. These cysticercoids belong to the type “floricerceus” according to their morphology (Bondarenko and Kontrimovichus 1976). In the form of larva and hooks, however, they resemble the cysticercoid of *Aploparaksis filliformis* Spassky, 1961. Although the identification of the cysticercoids has not been experimentally verified by the author, we assume that the larva cannot be assigned to the species *W. cirrosum*.

The intermediate host of *W. chaunense* is the oligochaete *Rhyaccdrilus coccineus*, which corresponds to the ecology of the definitive host, *G. gallinago*. The cysticercoid differs in its morphology from the above-mentioned cestodes of this genus developing in crustaceans and may be ascribed to the type “ramicerceus”. The single difference is that in the typical ramicercus (Bondarenko and Kontrimovichus 1976) the basic stem of tail is much longer than the appendages, whereas in *W. chaunense* all tail appendages are of almost the same length.

We have observed closing of lacuna primitiva during the development of *W. chaunense*, but according to many papers elucidating the development of the ceroecyst, the walls of this cavity transform into a cyst in which the scolex and neck move after the scolexogenesis is completed. In *W. chaunense* the cyst cavity is newly formed at the stage of scolexogenesis.

While studying the larvogenesis (ramicerceus type) of *Aploparaksis orientalis* Spassky et Bobova, 1961, parasite of *G. gallinago*, in the body of the oligochaete *Lumbriculus variegatus* we realized the identity of this process with that described for *W. chaunense* (unpublished data).

Besides the crustaceans also the oligochaetes may thus serve as intermediate hosts of *Wardium* cestodes (the participation of Hirudinacea should be verified). There are differences in the morphology and process of the post-embryonic development in larvae of different species. The same concerns the cestodes of the genus *Aploparaksis* Clerc, 1903, in which we observed a distinct polymorphism of larvae, though the oligochaetes served as intermediate hosts in all cases (Bondarenko and Kontrimovichus 1976). The life-cycles of these cestode genera are very interesting, but further studies are necessary to arrive at a conclusion.
РОСТ ОМБРИОНАЛЬНОЕ РАЗВИТИЕ ЦЕСТОДЫ WARDIUM CHAUNENSE BONDARENKO ET KONTRIMAVICHUS, 1977 (HYMENOLEPIDIDAE) В ПРОМЕЖУТОЧНОМ ХОЗЯИНЕ

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Development of *Wardia chaunense*. Fig. 1. Growing oncosphere. Fig. 2. Elongated larva.
Development of *Wardium chaunense*. Fig. 1. Scolexogenesis, formation of capsule. Figs. 2-3. Completion of scolexogenesis, larva before invagination. Fig. 4. Young cysticercoid. Figs. 5-6. 32-day-old larva (general view and internal structure). Figs. 7-8. Mature cysticercoids (general view).