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HAPLOMETRA CYLINDRACEA (TREMATODA: PLAGIORCHIIDAE) IN *LYMNAEA TRUNCATULA*: CERCARIAL SHEDDING DURING SINGLE OR DUAL INFECTIONS WITH OTHER DIGENEAN SPECIES

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ABSTRACT: The daily cercarial production of *Haplometra cylindracea* was studied in *Lymnaea truncatula* naturally infected by this parasite. Observations were performed in preadult snails with developing infections and in adults shedding cercariae at the beginning of the experiment. In these two groups, the cercarial production was analysed in controls and in snails subjected to a second infection with another trematode species (*Fasciola gigantica, F. hepatica*, or *Paramphistomum daubneyi*). The cercariae of *H. cylindracea* were shed over a patent period of 32-47 days. Their mean numbers were maximal at days 4-6 of the patent period and regularly decreased until day 32; afterwards, they were low and irregular. No periodicity was noted in the distribution of the daily mean numbers of *H. cylindracea*. When the infected snails were exposed to a second digenean species, a more rapid decrease in the mean daily values of *H. cylindracea* was noted, with cessation of shedding from days 26 to 29 of the patent period (according to the group). Metacercariae of the other trematodes were formed from day 28 or day 35 of the patent period; their production lasted from 10 to 19 days. However, the daily number of these parasites remained low (1-8 per snail).

KEY WORDS: Trematoda, *Haplometra cylindracea, Fasciola gigantica, F. hepatica, Paramphistomum daubneyi, Lymnaea truncatula*, cercarial production, single and dual infections.

INTRODUCTION

As well known, the molluscan species *Lymnaea truncatula* Müller, 1774 (Basommatophora: Lymnaeidae) plays the role of intermediate host of *Fasciola hepatica* Linnaeus, 1758 (Trematoda: Fasciolidae) in Europe. But moreover this snail species also acts as intermediate host in the life cycle of many other digenean species. This capacity to be used by trematodes pertaining to different digenean families raises several questions concerning the cercarial shedding patterns shown by these different digenean species in the same snail species, not only in the cases in which the snail individuals are parasitized by the larval stages of only one digenean species, but also in those cases in which larval stages of two different digenean species coexist within the same molluscan individual.

Concerning single infections, worth mentioning are the differences found between two different species of the same digenean genus. Thus, most cercariae of *Fasciola hepatica* are shed by *Lymnaea truncatula* at several times (shedding waves) of variable length, separated by intervals without shedding. Two rhythmic cycles can be recognized in these cercarial sheddings: an infradian-type rhythm with periodicity every 7 days, and a circadian rhythm with maximal production of parasites during the night (AUDOUSSET *et al.*, 1989). The latter rhythm is also found in the cercarial shedding of *Fasciola gigantica* Cobbold, 1855 in the same snail species; however, there is no daily periodicity (RAKOTONDRAVAO & RONDELAUD, 1991).

Concerning dual infections, one has to bear in mind the well known antagonism between digenean species at molluscan level (LIM & HEYNEMAN, 1972; COMBES, 1982). Among the numerous aspects of the intramolluscan inter-trematode antagonism, the aim of this paper is to contribute, by means of the model digeneans/*L. truncatula*, in two questions. Does dual infection of the snail affect the chronology of cercarial shedding in the first parasite species? Are changes in cercarial shedding similar when the second infection is performed during the parthenitae development of the first parasite or at the beginning of its cercarial shedding? To answer the former question, the species *Haplometra cylindracea* (Zeder, 1800) Looss, 1899 (Trematoda: Plagiorchiidae) has been used, since *L. truncatula* is a natural intermediate host in this trematode's life cycle (COMBES, 1968; GRABDA-KAZUBSKA, 1970). Cercarial shedding of this parasite was analysed in naturally infected *L. truncatula*, using controls and snails subjected to a second infection with another trematode species. For this purpose, *F. hepatica*, *F. gigantica*, or *Paramphistomum daubneyi* Dinnik, 1962 (Paramphistomatidae) were used. To answer the latter question, we studied preadult *L. truncatula* with developing infections with *H. cylindracea*, and adult snails shedding cercariae at the beginning of the experiment.

MATERIAL AND METHODS

Snails

L. truncatula specimens were collected at La Basse-Mazelle, between the communes of Limoges and Rilhac-Rancon, department of Haute-Vienne, France. They were living in a stream that runs throughout the year from the exit point of a subterranean drainage system collecting water of several springs. This colony was widely infected by *H. cylindracea*, with prevalences from 37% to 78% (HOURDIN, MOUKRIM & RONDELAUD, 1991). The superficial sediment of the habitat was siliceous (water pH: 6,5). Preadult snails (4 mm in height) and adults (5 to 5,5 mm) were used in these experiments. One hundred and thirty preadults were collected in June, and were part of the second annual generation. They harboured natural infection by *H. cylindracea*. Histological examination of 35 other preadult snails gathered in the same conditions, bred in laboratory, and killed from day 3 to day 42 after collection demonstrated that natural infection by *H. cylindracea* had occurred 15 days before snail collection in their habitat (MOUKRIM, HOURDIN & RONDELAUD, 1991). Three hundred and seventy-five adults were collected in April and were part of the first annual generation. They were infected by *H. cylindracea* and were shedding cercariae at the time of their collection. Histological examination of 35 adults using the same protocol revealed that the span of haplometrid snail infection was 30-35 days at the time of their collection (MOUKRIM & RONDELAUD, 1992). Snails were transported to the laboratory under isothermal conditions and kept in standard breeding containers for 48 h (at 20° C) before being subjected to experimentation.

Parasites

Three trematode species were used: *F. hepatica, F. gigantica*, and *P. daubneyi*. Eggs of *F. hepatica* were collected at the Limoges slaughterhouse (Haute-Vienne, France) and were collected in heavily-infected bovine gallbladders. Eggs of *F. gigantica* originated from gallbladders of Malagasy infected cattle. Eggs of *P. daubneyi* were collected in faeces of French cattle only infected with this helminthosis. All eggs were maintained for 16 days at 23° C in total darkness, in accordance with the reports of OLLERENSHAW (1971) concerning *F. hepatica*.

Experimental protocol

The first 45 preadults served as controls. The 85 others were each exposed to 2 miracidia of *P. daubneyi* for 4 h in 35-mm diameter Petri dishes containing 2 ml of water from their original habitat. The snails were bred in closed-circuit aquaria in an air-conditioned room at constant temperature (20° C), with a 12-h photophase and light intensity of 4000 lux at the container's surface. The snails were fed lettuce *ad libitum*. On day 35, the survivors were isolated in 35-mm Petri dishes with 2 or 3 ml of water and a piece of lettuce. The dishes were placed in a room which was open to the outside, but was protected from direct sunlight. The mean temperature was 22° C with extremes of 13° C and 25° C. Every morning the cercariae were counted, whether alive or dead. The water was changed daily and lettuce added as needed. The snails were observed until their death.

The first 45 adults served as controls. Half of the 330 others were exposed to *F. hepatica* (two miracidia per snail) and the other half to *F. gigantica* according to the same protocol. Miracidial exposure occurred the day that the first *H. cylindracea* cercariae were shed. From the beginning of the experiment, the snails were isolated in 35-mm diameter Petri dishes. Daily observations and cercarial counts were performed identically as with the preadults.

H. cylindracea cercariae were «active cercariae» and did not encyst on container walls. Those from other trematodes formed metacercariae after several minutes.

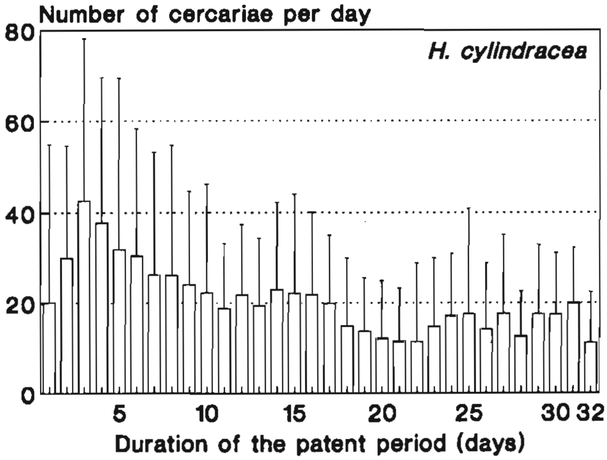

Fig. 1.— The number of cercariae shed by preadult controls in relation with the days of the patent period.

Processing of data

Figures concerning cercarial counts were all averaged and s.d. determined, taking into account the numerical order of the days of the patent period. Daily results were processed using an autocorrelation analysis (BROOM, 1979) to determine whether there was periodicity in the production of cercariae.

RESULTS

Preadult controls

There were 31 survivors on day 42, with a mean shell height of 8,2 mm. Cercarial shedding occurred in 28 snails. The beginning of the patent period occurred from days 35 to 38 of the experiment for 27 snails, and at day 51 for the last snail; its maximum duration was 32 days. The number of snails decreased rather slowly until day 27 of the patent period, with seven deaths during this phase; a sudden numerical decrease then occurred until day 32 (data not shown). One shedding wave was noted only in eight snails. Sheddings occurred 2, 3, 4, and 5 times respectively in 6, 3, 9, and 2 snails. Wave duration was 13,7 ± 10,4 days for the first wave, 6 ± 5,4 days for the second wave, and 3 to 3,5 days for the last three waves. The interval between waves was most often one day, sometimes two days (data not shown).

Daily counts of cercariae demonstrated an increase in mean numbers until day 3 and a constant, daily decrease until day 32 of the patent period. At this date, the mean number of cercariae shed was 11,2 (Fig. 1). No production rhythm was noted (data not shown).

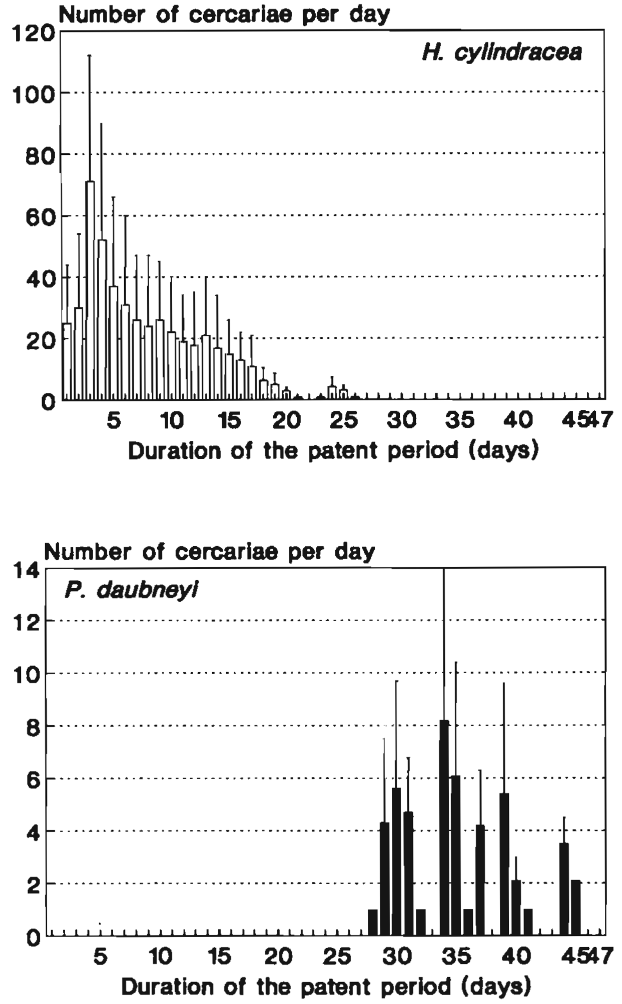

Fig. 2.— The number of cercariae shed by preadult snails infected by *H. cylindracea-P. daubneyi* in relation with the days of the patent period.

Preadults infected by *H. cylindracea* and *P. daubneyi*

There were 14 survivors at day 42, with a mean shell height of 8,6 mm. Twelve had cercarial sheddings. The beginning of the patent period was identical to the figures previously reported in corresponding controls; its maximum duration was 45 days. Four deaths were observed during the first seven days of the patent period. The number decreased more slowly until day 45 (data not shown). The number of waves of cercarial shedding was also identical to the figures reported in preadult controls.

H. cylindracea shedding was maximal at day 3, with 72 cercariae per snail. Afterwards, there was a constant decrease in mean numbers until day 21 of the patent period, and shedding ceased on day 26 (Fig. 2a). Only three snails shed *P. daubneyi* cercariae: the first cercarial shedding began at day 28 of the patent period and lasted 16 days; the daily number was low, from 1 to 8 parasites per snail (Fig. 2b).

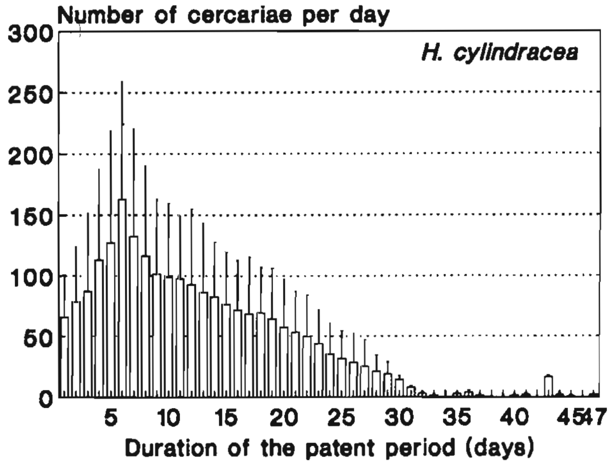

Fig. 3.— The number of cercariae shed by adult controls in relation with the days of the patent period.

Adult controls

Observations began on the first day of the experiment, i.e. the first day *H. cylindracea* cercariae were shed. The mean size of survivors was 9,3 to 9,7 mm at day 42. These snails survived longer than the corresponding preadults, with a maximal duration of 47 days for patent period. The number of snails decreased slowly until day 21 of the experiment; it subsequently decreased more rapidly until the end of the observation period (data not shown). There were 1, 2, 3, 4, 5, or 6 waves in cercarial shedding. Wave duration depended on numerical order and yielded figures which were similar to those of the corresponding preadults. The interval duration between waves differed: it lasted 1 or 2 days if there were 1 or 2 waves, and could last 11, 14, and 17 days for the others (data not shown). Twenty-one snails died after 2 to 7 days following the last cercarial shedding.

Cercarial production was maximal at day 6 of the patent period and decreased constantly until day 33. Shedding was subsequently intermittent until day 47 and the production of cercariae remained low (not more than 8,3 cercariae per snail) (Fig. 3). No rhythm in parasite sheddings was demonstrable (data not shown).

Adults infected by *H. cylindracea* and *Fasciola* sp.

The results of the two groups were partially similar. The mean size of survivors was 9,3-9,7 mm at day 42 of the experiment. Snail number decreased suddenly during the first 7 days, with 45% of dead snails in the *F. hepatica* group, and 54% in the *F. gigantica* group. Afterwards, the numerical decrease was slower. The last survivors were seen at day 47 in the former, and at day 45 in the latter (data not shown).

Shedding characteristics differed according to the trematode species. Wave number and duration noted for *H. cylindracea*, and the interval between waves, were similar to corresponding controls. The daily number of

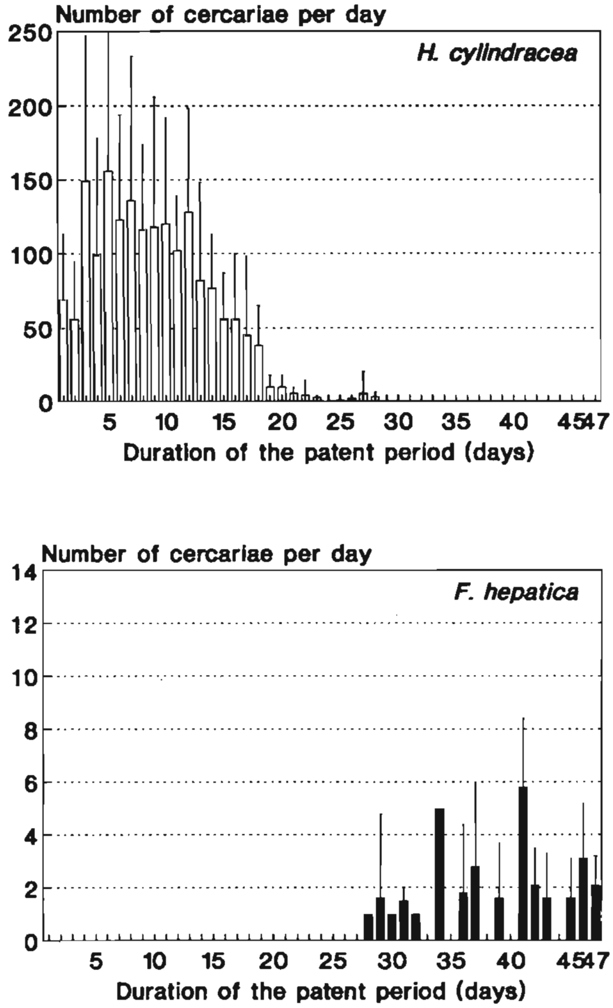

Fig. 4.— The number of cercariae shed by adult snails infected by *H. cylindracea-F. hepatica* in relation with the days of the patent period.

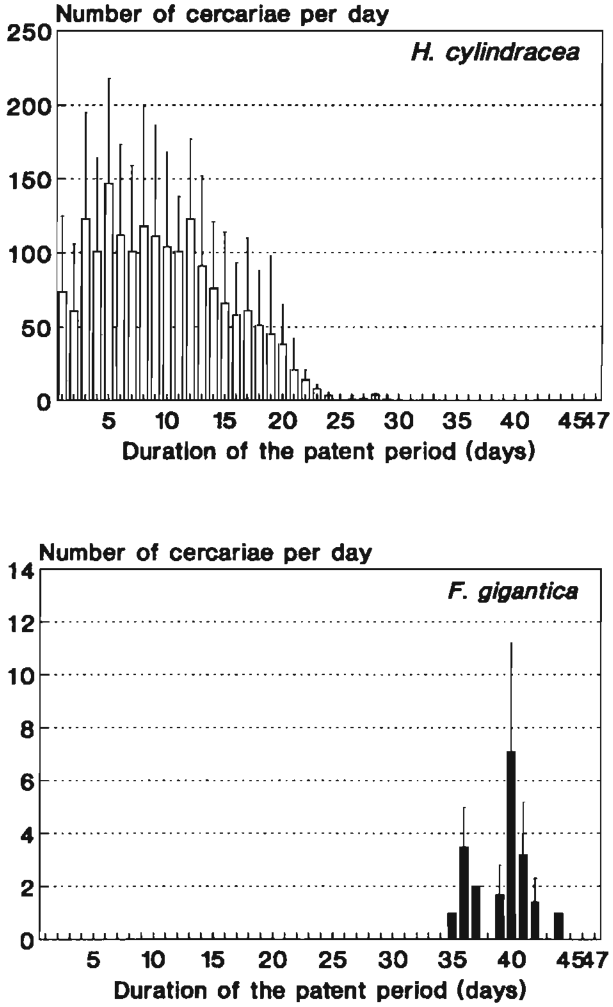

Fig. 5.— The number of cercariae shed by adult snails infected by *H. cylindracea-F. gigantica* in relation with the days of the patent period.

cercariae was maximal on day 5 of the patent period. The mean number subsequently decreased at a constant rate until days 23 or 25. Later sheddings were irregular and disappeared at days 28 or 29 (Fig. 4a and 5a).

Seventeen snails in the *F. hepatica* group shed cercariae and 7 in the *F. gigantica* group. Their first shedding began at day 28 of the patent period in the first group (Fig. 4b), at day 35 in the other (Fig. 5b). The shedding lasted over a period of 19 days in the *F. hepatica* group, and 10 days in the other group. Intervals between waves were quite frequent and of rather brief duration. The daily number of cercariae shed per snail was low, and did not exceed 7 parasites (Fig. 4b and 5b).

DISCUSSION

No daily periodicity was noted in cercarial sheddings of *H. cylindracea*. This fact can only be interpreted in the light of COMBES' review (1983). Cercariae from this species are «active» and search for their definitive host (in this case, *Rana temporaria* tadpoles) before penetrating and encysting (GRABDA-KAZUBSKA, 1970). Since this definitive host is in an aquatic environment until its metamorphosis into a young frog, the shed cercariae are in continuous contact with their host, which could explain why no rhythmic pattern was noted in this trematode.

There were no reports in the literature on dual infection characteristics when a second exposure was performed at the beginning of cercarial shedding from the first parasite. Comparison of our results between preadults and adults shows that infection of *L. truncatula* by a second trematode did not influence the chronology of cercarial shedding in *H. cylindracea* until days 12-15 of the patent period; afterwards, there was a sudden decrease in cercarial numbers and the shedding ceased at days 26-29. A discordance can be noted between results in the two snail groups: experimental decrease in *H. cylindracea* sheddings began from days 47-50 in the preadult group and from days 14-15 in the adult group. There is no satisfactory explanation for the difference between snail groups. The most likely hypothesis seems to be that this difference may be related to the second parasite species. As sporocysts of *H. cylindracea* and rediae of the other trematode were located in separated compartments of snail body until day 28 of the experiment (MOUKRIM, HOURDIN & RONDELAUD, 1991; MOUKRIM & RONDELAUD, 1992), it might be thought that secretions or excretions of *Fasciola* rediae would be more active on involution of *H. cylindracea* sporocysts than those of *P. daubneyi* rediae and would act more speedily by stopping the emergence of independent cercariae from sporocysts or their shedding from snails. This hypothesis is partly based on results given by histological examination of sporocyst burden in these snails: the development of *P. daubneyi* in preadult snails induced progressive involution of *H. cylindracea* sporocysts and granuloma formation (MOUKRIM, HOURDIN & RONDELAUD, 1991), whereas *Fasciola* development in adults induced only a sporocyst involution (MOUKRIM & RONDELAUD, 1992).

The dual infection of *L. truncatula* by *P. daubneyi* was manifested by cercarial shedding at day 28 of the patent period, which corresponded to days 63 or 65 of the experiment. These findings disagree with our knowledge of individual snail infection by the same parasite (POSTAL, unpublished thesis, Alfort, France, 1984; MOUKRIM, unpublished thesis, Agadir, Morocco, 1991), where snail death occurs without shedding or with heavy production in a single day together with the host's death, which could favour the host's inadaptability to its parasite. The duration of *Paramphistomum* shedding noted in dual infections (10 days) and the number of shed parasites can be interpreted as a facilitating effect (COMBES, 1982) that *H. cylindracea* develops against *P. daubneyi*. The histopathological abnormalities previously cited in these dually-infected snails (MOUKRIM, HOURDIN & RONDELAUD, 1991) may account for this facilitating effect.

The results of the dual infections with *Fasciola* species agree with the observations by other authors concerning cercarial shedding in dually-infected snails (LIM & HEYNEMAN, 1972; COMBES, 1982; CHIPEV, VASSILIEV & SAMNALIEV, 1985; HATA *et al.*, 1988; HOURDIN, RONDELAUD & CABARET, 1990). The only point which differs concerns the rapid cercarial production of *Fasciola* species from day 28 or day 35 of the experiment (at 20° C). From these results, we set forth the hypothesis that there was no longer a balanced host-parasite relationship (SCHWANBECK, BECKER & RUPPRECHT, 1986) in dually-infected snails when cercarial shedding from the first parasite occurred. This absence of regulation would favour growth of the second trematode, resulting in the development and accelerated maturation of one or several rediae. Additional studies are needed to verify this hypothesis by comparing sporocyst and redial maturity in infected snails according to the same protocol, however, with one miracidium per trematode species present.

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