ENCYSTMENT SITES OF METACERCARIAE OF *HYPODERAEUM CONOIDEUM* (TREMATODA: ECHINOSTOMATIDAE) IN FRESHWATER GASTROPODS

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Hypoderaeum conoideum (Bloch, 1782) is a widespread parasite of fowls and mammals, including man. Species of Lymnaeidae (Gastropoda) have been recorded as first intermediate hosts, with snails and tadpoles serving as second intermediate hosts. In Spain, *Lymnaea peregra* (Müller, 1774) was reported as natural first intermediate host (Toledo R., Muñoz-Antolí C., Pérez M., Esteban J.G. 1998: J. Helminthol. 72: 79-82). The life-cycle was first described by Mathias (Mathias P. 1925: Bull. Biol. Fr. Belg. 59: 1-125), though detailed information on the biological characteristics of each stage is not available. Some studies have revealed an interesting pattern in the behaviour of the *H. conoideum* cercariae (Haas W., Haberl B., Kalbe M., Körner M. 1995a: Parasitol. Today 11: 468-472; Haas W., Körner M., Hutterer E., Wegner M., Haberl B. 1995b: Parasitology 110: 133-142). In this sense, it would be interesting to determine how this behaviour may influence metacercarial establishment in the second intermediate host.

Cercariae of *H. conoideum* were obtained from a pool of naturally infected specimens of *L. peregra* collected in the Albufera Natural Park (Valencia, Spain). Laboratory-reared specimens of *L. peregra*, *L. palustris* (Müller, 1774) (Lymnaeidae), *Physa acuta* (Draparnaud, 1805) (Physidae), and *Gyraulus chinensis* (Dunker, 1845) (Planorbidae) were used as experimental second intermediate hosts. Thirty specimens of each gastropod species were exposed individually for 1 hr to 30 freshly emitted (maximum age 30 min) cercariae in 10 ml of spring water at 20 ± 1ºC. All snails exposed to infection were in the length range 0.3-0.5 cm**.** After 48 hr post-exposure, all the soft tissues of the snails were examined for encysted metacercariae.

A total of 117 (98%) of the 120 experimentally exposed snails were infected, and a total of 1,869 metacercariae (infection intensity: 15.6 ± 1.6 cysts per host) were recovered. Prevalence of infection and infection intensity varied according to the snail species (*L. peregra*: 93% and 14.0 ± 1.6, respectively; *L. palustris*: 100% and 15.3 ± 1.3; *P. acuta*: 97% and 13.0 ± 1.4; and *G. chinensis*: 100% and 20.2 ± 1.4). Although the differences in the percentage of snails infected among these snail species were not significant, analysis of variance using cyst counts transformed by log (x+1) revealed significant differences between the numbers of cercariae establishing as cysts in each of these hosts ($P (F = 3.78) < 0.01$). A *t*-test on cysts counts transformed by log (x+1) indicated that the number of cysts established in *G. chinensis* was significantly greater than both *L. peregra* ($P < 0.01$) and *P. acuta* ($P < 0.005$).

Variable numbers of metacercariae were found in the lumen of the pericardial cavity and kidney. No other organs or tissues were found to be involved in the metacercarial encystment. Pericardial cavity was the most selected site in all the snail species (Fig. 1). The *t*-test comparison on cysts counted in each encystment site showed the number of metacercarial cysts detected in the pericardial cavity to be significantly higher than in the kidney lumen in all infected snails ($P < 0.01$) and for each species (*L. peregra, P. acuta* and *G. chinensis*: $P < 0.01$ and *L. palustris*: $P < 0.05$). No dead or deformed metacercariae were observed.

The echinostome cercariae penetrate in the snail second intermediate host through the natural body openings. According to Anderson and Fried (Anderson J.W., Fried B. 1987: J. Parasitol. 73: 49-54) and Lo (Lo C.T. 1995: J. Parasitol. 81: 569-576) the most important entry is via the nephridiopore, which results in encystment mainly in the kidney lumen and secondarily in the pericardial region by migration from the kidney by the renopericardial canal (Najarian H.H. 1954: J. Morphol. 94: 165-197). The results obtained here show that the pericardial cavity is the most selected site of metacercarial encystment for *H. conoideum* and fewer metacercariae were detected in the kidney lumen. The absence of metacercariae encysted in other locations suggests that *H. conoideum* cercariae only penetrate the snail via the nephridiopore. Information on the mechanisms of site location is scarce, though they could be related to the chemical cues emitted by the snails. Anderson and Fried (1987, op. cit.) suggested that the excretory products emitted from the snail's nephridiopore are probably involved in the site finding of echinostome cercariae. However, other data on the metacercarial encystment sites suggest that other products are also involved in this process. In this sense, the preference for access to the snail via its nephridiopore shown by *H. conoideum* cercaria could be related to the nature of the stimulating cues in the host-finding processes. Haas et al. (1995a, b, op. cit.) demonstrated that *H. conoideum* cercariae are attracted in the host-finding processes by chemical cues different to those of other echinostome cercariae. This fact could account for the different entry route in the snail and could explain the behaviour shown by this cercaria. The involvement of the pericardial cavity as the major site of metacercarial encystment has not previously been reported in

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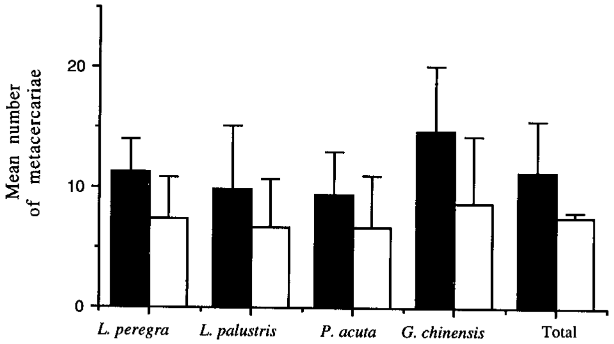

Fig. 1. Mean number of *Hypoderaeum conoideum* metacercariae in the sites of encystment for each *Lymnaea*, *Physa* and *Gyraulus* species and for the entire sample. The vertical bars are the values of the standard deviation. (■) pericardial cavity; (□) kidney lumen.

other echinostomes. Several echinostome cercariae, e.g., *Echinoparyphium flexum* (Najarian H.H. 1954, op. cit.), *Echinostoma trivolvis* (Anderson J.W., Fried B. 1987, op. cit.) (referred to as *E. revolutum* in their study), and *E. macrorchis* (Lo C.T. 1995, op. cit.), encyst mainly in the kidneys, while in others species, e.g., *Echinoparyphium ellisi* (Johnston T.H., Angel L.M. 1949: Rec. S. Aust. Mus. 9: 247-254), *E. baguali* (Jain G.P. 1960: Parasitology 61: 123-126), and *E. recurvatum* (Adam M.E., Lewis J.W. 1992: J. Helminthol. 66: 96-99), the mantle cavity has been reported as the prime site of encystment. This fact suggests that these last echinostome cercariae preferentially penetrate the snail via pneumostome.

Detailed studies on the transformation of cercariae to infective metacercariae are not available (Dönges J. 1969: Z. Parasitenkd. 31: 340-366). However, Lo (1995, op. cit.) suggested that the encystment occurs during the cercarial migration within the snail and could be determined by the time required for the cyst formation in each digenean species. In this sense, we consider that the developmental time for the transformation of *H. conoideum* cercariae into infective metacercariae within the snail should be explored further.

The absence of dead and deformed metacercariae observed in the present study is in agreement with other authors (Evans N.A., Gordon D.M. 1983: Z. Parasitenkd. 69: 217-222; Adam M.E., Lewis J.W. 1992, op. cit.) and, together with the prevalences and intensities of infection obtained, could indicate a high suitability of the snail species exposed to cercariae as second intermediate hosts for *H. conoideum*. This fact may contribute to the enhancement of the transmission of this parasite in the studied area, where these are the most frequently occurring freshwater snails (Toledo et al. 1998, op. cit.). This broad specificity increases the density of potential hosts in a given habitat and moreover contributes to protect the parasite from the deleterious effects of any decline in the local population of the molluscan host species. However, the degree of compatibility shown by *H. conoideum* cercariae to their natural and experimental first intermediate host, *L. peregra*, in our study differs from data reported for other echinostomes, such as *Pseudoechinoparyphium echinatum* (McCarthy A.M., Kanev I. 1990: Parasitology 100: 423-428) where cercariae develop a low degree of compatibility toward their respective first intermediate host - *Lymnaea stagnalis*. This pattern is recognised as a mechanism preventing metacercarial superinfection of the emitting host, and also preventing parasite pressure on the first intermediate host population (McCarthy A.M., Kanev I. 1990, op. cit.). Nevertheless, our results suggest that the high compatibility shown by *H. conoideum* cercariae to different gastropod species that co-exist with the parasite in the same natural habitat compensate this fact.

Acknowledgements. The present study was supported by Spanish DGICYT Projects PB92-0517 and PB96-0401-C02-02 of the Ministerio de Educación y Ciencia, Madrid. Thanks are also due to the Direcció General de la Conselleria de Medi Ambient de la Generalitat Valenciana (Valencia, Spain) for the licence to collect host materials in the field. This work was done whilst the second author (C.M.A.) was in receipt of a pre-doctoral fellowship from the Conselleria d'Educació i Ciència de la Generalitat Valenciana (Valencia, Spain).

Received 7 April 1998 Accepted 8 July 1998