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# ANTAGONISTIC INTERACTION BETWEEN ECHINOSTOMA REVOLUTUM AND ECHINOPARYPHIUM RECURVATUM (TREMATODA) IN THE DEFINITIVE HOST

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**Abstract.** The results are presented of experimental studies on the requirements of the microhabitat and antagonistic interactions between two trematode species (*Echinostoma revolutum* and *Echinoparyphium recurvatum*) in the small intestine of laboratory hamsters. It was found that antagonistic interactions are manifested mainly in the adult worms as a form of interactive site segregation. The co-existence of both fluke species in separate microhabitats of the small intestine was apparent.

In recent years, the problem of interspecific interaction between helminths has received increased attention. The surveying, theoretical analysis of this problem given by Holmes (1973), with a classification of the phenomenon and its different categories constitutes the most detailed account noted in the literature. The solution of these problems has been based mainly on a statistical evaluation of natural infections, less frequently on experimental studies.

For these reasons, our approach to this problem has been made by way of experimental studies on the interaction of two closely related fluke species (*Echinostoma revolutum* and *Echinoparyphium recurvatum*) in the organism of the definitive host. Our investigation led by the path described in this paper, to the demonstration in the laboratory of specific site selection by these flukes in the organism of the definitive host infected with a single species and of their interactions in simultaneous mixed infection with both species.

## MATERIAL AND METHODS

While studying in experiments the life cycles of *Echinostoma revolutum* (Froelich, 1802) and *Echinoparyphium recurvatum* (Linstow, 1873), we obtained a large number of cercariae of these trematodes (Moravec et al. 1974). We set up two groups of snails, *Biomphalaria alexandrina* (Ehrenberg), and exposed them to one of the pertinent cercarial species. Encysted metacercariae from the snails were used for infection of laboratory hamsters *Mesocricetus auratus* (Waterhouse). Each definitive host was fed with metacercariae aged 8—10 days; the number of metacercariae per infective dose was determined by means of examination of the snails with a compressorium. We infected a total of 32 hamsters aged 5—6 months. Of these we formed four experimental groups and infected the snails of each group in the following way: group 1 (5 hamsters with a single *E. revolutum* infection, dissection on day 17 p.i., infective dose approximately 200 metacercariae); group 2 (7 hamsters with a single *E. recurvatum* infection, dissection on day 17 p.i.; infective dose approximately 200 metacercariae); group 3 (10 hamsters with a simultaneous mixed infection of metacercariae of *E. revolutum* and

*E. recurvatum*, dissection, on day 10 p.i., infective dose 200 + 200 metacercariae of each species); group 4 (10 hamsters with simultaneous mixed infection of *E. revolutum* and *E. recurvatum* metacercariae, dissection on day 17 p.i., infective dose 100 + 100 metacercariae of each species). The infective dose in group 4 was lower than that in group 3 in order to reduce host mortality which was high in the latter group following exposure to 400 metacercariae per host. The individual experimental groups were kept separately, fed with sterilized, standard food and additional vegetables.

The results of infection were determined by dissection. After removal of the digestive tract, the small intestine was spread out in order to determine its overall length. Then it was divided into 10 equally long sectors and the number of trematodes was recorded separately for each sector. The two trematode species could be distinguished with the stereoscope; for the assessment of the exact number of collar spines, we examined several specimens with the microscope. The trematodes were fixed for studies on their biometry and morphological variability. Also the remaining organs were dissected individually. Hosts, which died during the course of the experiments, were not included in the evaluation of the results of infection and localisation. This applied to 7 out of the total number of 32 infected hamsters.

## RESULTS

**Group 1** (single infection with *E. revolutum* on day 17. p.i.): We dissected 4 hamsters (out of the total number of 5 hamsters infected, one animal died during the experiment); the average number was 158.4 *E. revolutum* specimens in one host. The trematodes were distributed along the length of the inspected sectors of the small intestine (a total of 140.8 flukes per host) and also in the large intestine (an average of 17.6 flukes per host). The largest number of trematodes was found in the third tenth of length of the small intestine; at the average length of the small intestine of hosts in this group of 32.8 cm the highest concentration of flukes occurred at 6.5—9.8 cm from the anterior end. An evalution of the complete set showed clearly that, in a single infection, this species occupied predominantly the anterior half of the small intestine (Fig. 1), but colonized in small numbers also other sections of the small and large intestine. The average worm load in the hamster was 79 % (out of the original infective dose), of this 70.4 % in the small intestine, the rest in the large intestine.

**Group 2** (single infection with *E. recurvatum*, on day 17 p.i.): An evaluation of the localisation of this species in 7 hamsters disclosed a marked preference for the anterior half of the small intestine. The average number of worms per host was 125.6 flukes. No flukes were found in the large intestine. The average worm load in the host was 62.8 %, which was lower than that in hosts of the foregoing group (single infection with *E. revolutum*). The average length of the small intestine of hamster in this group was 37.5 cm, the flukes of *E. recurvatum* were localized at 6/10th of its length (i.e. at 22.4 cm from the anterior end). They dominated in the third tenth of overall intestinal length, i.e., within 7.5—11.2 cm from its anterior end.

**Group 3** (mixed simultaneous infection with *E. revolutum* and *E. recurvatum* on day 10 p.i.): In the five surviving hamsters of this group (five out of ten hamsters died during the experiment) we found a quantitatively balanced double infection with both trematode species. The average number of flukes per host was 153.7 speciemens of *E. revolutum* and 149.5 specimens of *E. recurvatum*. *E. revolutum* was present mainly in the small intestine (151 specimens), several specimens were found in the large intestine (2.7 flukes). Both fluke species were sexually mature. Along the entire length of the small intestine (its average length was 33.0 cm) we observed first signs of separation of the two species (Fig. 3). *E. recurvatum* preferred, evidently, the anterior half of the small intestine, *E. revolutum* shifted to its posterior half. The largest number of *E. recurvatum* flukes was found in 2/10th of length of the small intestine, i.e., at 3.3 to 6.6 cm from its anterior end. *E. revolutum* dominated in 8/10th of length, i.e., at 23.1—26.4 cm from the anterior end of the small intestine. The average worm load with *E. revolutum* was 76.8 % (75.5 % in the small intestine), that of *E. recurvatum* 74.5 %.

**Group 4** (mixed simultaneous infection with *E. revolutum* and *E. recurvatum*, on day 17 p.i.): We evaluated 9 hamsters (one of the 10 infected animals died during the experiment), and found an average of 77.6 trematodes of *E. revolutum* in one host; of this

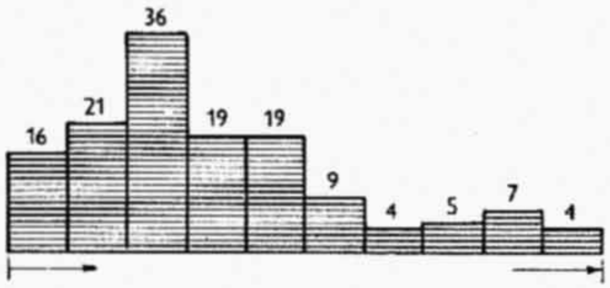


**Fig. 1.** Quantitative incidence of the trematode *Echinostoma revolutum* in the small intestine of laboratory hamsters on day 17. p.i (group 1). The columns of the histogram show 10 equally long sections of the small intestine, the numbers indicate the average incidence of flukes (rounded off).

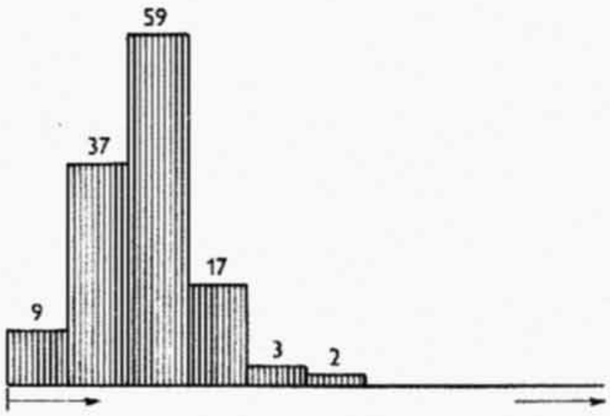


**Fig. 2.** Quantitative incidence of the trematode *Echinoparyphium recurvatum* in the small intestine of laboratory hamsters on day 17 p.i. (group 2). The columns of the histogram show 10 equally long sections of the small intestine, the number indicates the average incidence of flukes (rounded off).

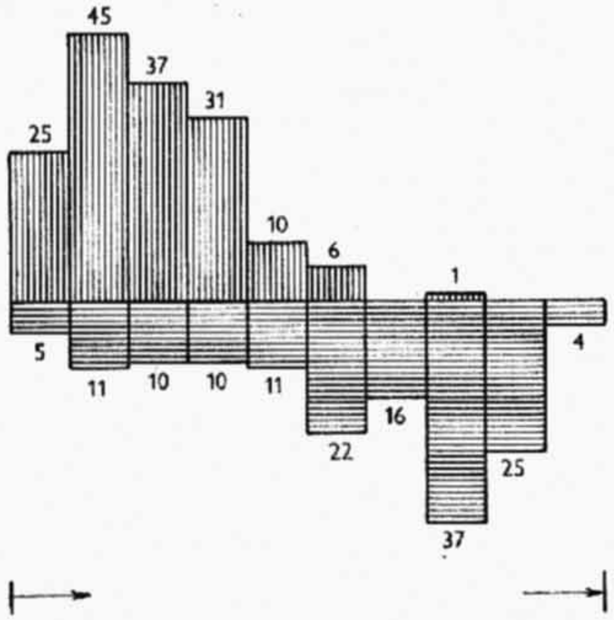


**Fig. 3.** Quantitative incidence of the trematodes *E. revolutum* and *E. recurvatum* in the small intestine of laboratory hamsters on day 10 p.i. (group 3). Upper part of the histogram for *E. recurvatum*, lower part for *E. revolutum* (average number of flukes rounded off).

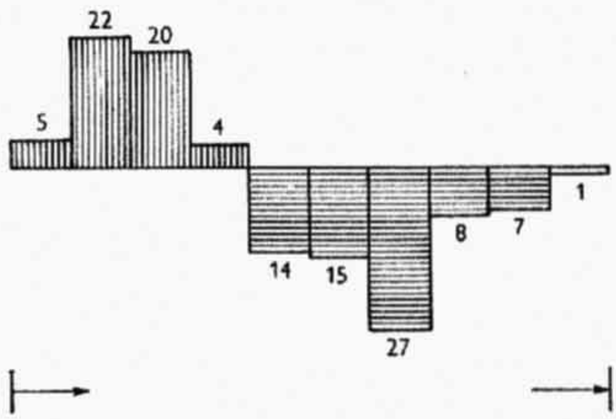


**Fig. 4.** Quantitative incidence of the trematodes *E. revolutum* and *E. recurvatum* in the small intestine of laboratory hamsters on day 17 p.i. (group 4). Upper part of the histogram for *E. recurvatum*, lower part for *E. revolutum* (average number of flukes rounded off).

5.7 specimens were present in the large intestine, the remaining worms in the small intestine. The average number of *E. recurvatum* was 51.3 flukes per host. The average worm load with *E. revolutum* was higher (77.6 %) than that with *E. recurvatum* (51.3 %).

The species *E. recurvatum* preferred evidently the anterior half of the small intestine (Fig. 4) predominantly the second and third tenth of this length, which in this group of hamsters attained 31.7 cm on the average; the localisation of these flukes extended to a distance of 12.6 cm from the anterior end of the small intestine. By contrast, *E. revolutum* was found in the fifth tenth of length of the small intestine, i.e., beyond the sites colonized by *E. recurvatum* (12.6 cm from the anterior end of the small intestine). A marked dominance of *E. revolutum* was observed in 7/10th of length of the small intestine (i.e., at 18.9 to 22.1 cm from its anterior end). In this case, the two fluke species colonized separate sections leaving always a short unoccupied space between the two species (1.3—2.5 cm)—(this is not visible in the figure because the sectors of the small intestine inspected and statistically evaluated were always longer—3.1 cm).

## DISCUSSION

The results of experimental infection of hamsters (*M. auratus*) with the flukes *E. revolutum* and *E. recurvatum* suggested that hamsters are highly susceptible definitive hosts. On day 17 p.i. the average worm load in the organism of the host ranged from 77—79 % for *E. revolutum*, 51—62 % for *E. recurvatum*. We observed remarkable differences between the two fluke species in their selection of specific sites (or microhabitats) in the organism of the definitive hosts. In comparison with *E. recurvatum*, the ecological valence of *E. revolutum* was wider; it colonized the entire small intestine (Fig. 1) and was found also in the large intestine. In a single infection, however, *E. revolutum* dominated in the anterior small intestine. *E. recurvatum* displayed a marked specificity in microhabitat selection to the anterior small intestine of its hamster-host (predominantly in the first fourth of its total length). It was not present in the remaining parts of the small intestine, and in the large intestine (Fig. 2). This finding is in accord with the data of Rašín (1933) obtained from experimental infection of the house pigeon (*Columba livia* f. *dom*); in his experiments, *E. recurvatum* always colonized the anterior small intestine. Although the flukes extended beyond this zone in case of heavy infection, this species displayed always a specificity to and dominance in the anterior small intestine. We should like to remark that our methods used for dissection and evaluation of the experiments were essentially similar to those employed by Rašín (1933).

The evaluation of concurrent double infections in groups 3 and 4 demonstrated antagonistic interactions between the two species. It appears that the separation of *E. revolutum* and *E. recurvatum* (or the expulsion of *E. revolutum* from sites of its dominant localisation—Fig. 3) occurs at the adult stage. This leads to a complete spatial separation of the two species in the small intestine (Fig. 4) as recorded by us on day 17 p.i. At this time *E. recurvatum* flukes remained at the site of their specific localisation, while *E. revolutum* colonized the posterior small intestine (and partly the large intestine). The species *E. recurvatum* displayed a marked specificity to its microhabitat in the organism of its host. *E. revolutum* (according to results in group 1) is apparently more adaptable to a wider range of sites than the former species. It seems that the wider ecological valence of *E. revolutum* and the higher specialisation of *E. recurvatum* were, in the final issue, responsible for the expulsion of *E. revolutum* from the anterior small intestine.

Following Holmes' (1973) evaluation of interspecific interactions between parasites, the situation encountered in our case may be classified as a case of interactive site segregation. In a double infection the final distribution of both fluke species (*E. recurvatum* and *E. revolutum*) in the intestine of the definitive host was the result of interactions between the two parasite species, in single infections, both *E. recurvatum* and *E. revolutum* colonized approximately the same sites; although site specificity was stricter in *E. recurvatum* than in *E. revolutum*, they both selected the same optimal microhabitat (the second and third tenth of length of the small intestine). By contrast to phenomenons such as competitive exclusion or genetically based selective site segregation which seem to be common in parasites, interective site segregation is less frequent in occurrence; present information available has been obtained mainly from analyses of interactions between parasites in naturally infected hosts (e.g. MacKenzie and Gibson 1970; Chappell 1969).

The results of our experimental studies demonstrate that, in spite of antagonistic interactions between the adult flukes *Echinoparyphium recurvatum* and *Echinostoma revolutum* reflected in interactive site segregation, the two parasite species can co-exist in the intestine of the definitive host. This finding is very important if the two echinostome flukes (*E. recurvatum* and *E. revolutum*) were to be used for the biological control of schistosomiasis of man in Egypt (Heyneman et al. 1972; Ryšavý et al. 1973). By employing a suitable definitive host it may be possible to achieve simultaneously an increase in populations of both echinostome trematodes and control, in this way, both *Schistosoma haematobium* and *S. mansoni*.

## ПРОЯВЛЕНИЕ АНТАГОНИЗМА МЕЖДУ *ECHINOSTOMA REVOLUTUM* И *ECHINOPARYPHIUM RECURVATUM* (*TREMATODA*) В ОКОНЧАТЕЛЬНОМ ХОЗЯИНЕ

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**Резюме.** Экспериментально изучались предъявляемые к микростации требования и антагонизм двух видов трематод (*Echinostoma revolutum* и *Echinoparyphium recurvatum*) в тонкой кишке лабораторных хомяков. Результаты показали, что между половозрелыми трематодами обоих видов возникают антагонистические отношения в виде взаимодействующего локального расселения (сегрегации). Было выявлено совместное существование обоих видов трематод в отдельных микростациях в тонкой кишке.

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