

## Study of *Euparyphium albuferensis* and *Echinostoma friedi* (Trematoda: Echinostomatidae) antagonistic interactions in the experimental definitive host

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### Summary

Single and concurrent infections of *Euparyphium albuferensis* and *Echinostoma friedi* in an experimental definitive host have been done to study possible antagonistic interactions in infectivity, habitat location and growth rates. Similar infection rates appeared in *E. albuferensis* single and concurrent infections. The infectivity rate of *E. friedi* in single infections (8 %) was statistically lower than in concurrent infections (17 %). Location of most *E. albuferensis* worms in single infections was at the first third of the small intestine (segment I) away from *E. friedi* located in segment II and III. In concurrent infections of both parasite species, although most worms remained in the same location as in single infections, some adults were together in segment II where only *E. friedi* showed a significant reduction of its body and gonadal measurements. These two echinostomatid species could develop its life-cycles using the same definitive host without a clear antagonistic interaction.

**Key words:** *Euparyphium albuferensis*; *Echinostoma friedi*; single infection; concurrent infection; definitive host; antagonistic interaction

### Introduction

*Euparyphium albuferensis* Esteban *et al.*, 1997 and *Echinostoma friedi* Toledo *et al.*, 2000 are two related species that co-exist in the same natural habitat, the Albufera Natural Park (Valencia, Spain), parasitizing rats as definitive hosts. Experimentally, the golden hamster *Mesocricetus auratus* is as a useful definitive host for both parasite species (Esteban *et al.*, 1997; Toledo *et al.*, 2000).

The interspecific interactions between closely related helminths in definitive hosts is a normal fact (Baruš *et al.*, 1974; Meece and Nollen, 1996). The mechanisms behind these competitions are difficult to explain. The most detail-

ed account in the literature is that of Holmes (1973) with a classification of the phenomenon and its different categories. Antagonistic interactions could have a cost over the normal habitat location of the adult worms and a negative implication over the normal growth rates.

Most concurrent infection work is concerned with parasite species belonging to the same genera (Iorio *et al.*, 1991; Meece and Nollen, 1996; Fried *et al.*, 1997; Nollen, 1997). The purpose of this work is to compare the infectivity, the habitat distribution and adult growth rates of two echinostomatid species of different genera, in single and concurrent experimental hamster infections, to search whether or not the existence of antagonistic interactions exists.

### Material and Methods

Encysted metacercariae of *E. albuferensis* or *E. friedi* were removed from the renal tissue and pericardial cavity of experimentally infected individual *Physella acuta* snails. We infected a total of 15 24-days old golden hamsters (*M. auratus*) that were necropsied 22 days post-infection (d.p.i.) in all three experimental groups: group A (5 hamsters with a single *E. albuferensis* infection; infective dose of 100 metacercariae); group B (5 hamsters with a single *E. friedi* infection; infective dose of 100 metacercariae); and group C (5 hamsters with a simultaneous concurrent infection of metacercariae of *E. albuferensis* and *E. friedi*; infective dose of 100 + 100 metacercariae of each species). Hamsters were given commercial food and water ad libitum.

At necropsy, the small intestine was removed, measured (mean value: 39.7 ± 0.8 cm) and divided into 3 equal segments (I, II and III) from the stomach to the caecum (mean value of each segment: 13.2 ± 0.3). The worms recovered in each segment were counted and some of them were stained in Grenacher's carmine and mounted in Canada

Balsam. Body length, mid-acetabular width and gonadal areas were measured in 5 specimens of each group. Chi-square test was used to compare the recovery rates and the Student's t-test was used between singly or concurrently worms growth to compare differences in worm body area and gonadal areas. Values of  $P < 0.05$  were considered as significant.

## Results

Results of infectivity in single and concurrent infections are summarized in Table 1. Infectivity rates of *E. albuferensis* were maintained without statistical differences in

single and concurrent infections (13 % and 14 %, respectively). Total infectivity of *E. friedi* in single infections was statistically lower than the infectivity rate found in concurrent infections (8 % vs 17 %) ( $P (\chi^2 = 21.2) < 0.05$ ; D.F.: 998).

The distribution of worms and the total percentage of worms in segments I, II and III in the small intestine of the three groups varied (Table 1). In single infections, most of the *E. albuferensis* worms (94 %) were located in segment I and only a few (6 %) were located in segment II with statistical differences ( $P (\chi^2=98) < 0.05$ ; D.F.: 126). None was recovered from segment III. Worms of *E. friedi* were recovered only from segments II (61 %) and III (39 %),

Table 1. Infectivity and microhabitat distribution of *E. albuferensis* and *E. friedi* adult worms in golden hamster (experimental definitive host)

| Group <sup>§</sup> | Parasite species         | No. cysts/host | Range space (X ± S.E.)<br>* worms recovered | Total No (%) worms recovered | No. (%) of worms in the small intestine (segments) |         |         |
|--------------------|--------------------------|----------------|---|------------------------------|--|---------|---------|
|                    |                          |                |   |                              | I  | II      | III     |
| A                  | <i>E. albuferensis</i>   | 100            | 7 – 22 (12.8 ± 6.8)                         | 64 (13)                      | 60 (94)  | 4 (6)   | 0       |
| B                  | <i>E. friedi</i>         | 100            | 3 – 14 (7.6 ± 4.6)                          | 38 (8)                       | 0  | 23 (61) | 15 (39) |
| C                  | <i>E. albuferensis</i> + | 100+           | 9 – 26 (13.8 ± 6.9)                         | 69 (14)                      | 61 (88)  | 8 (12)  | 0       |
|                    | <i>E. friedi</i>         | 100            | 10 – 21 (17.2 ± 4.3)                        | 86 (17)                      | 0  | 52 (60) | 34 (40) |

§ Groups A, B and C each with 5 hamsters; \*(X ± S.E.): mean value ± standard error

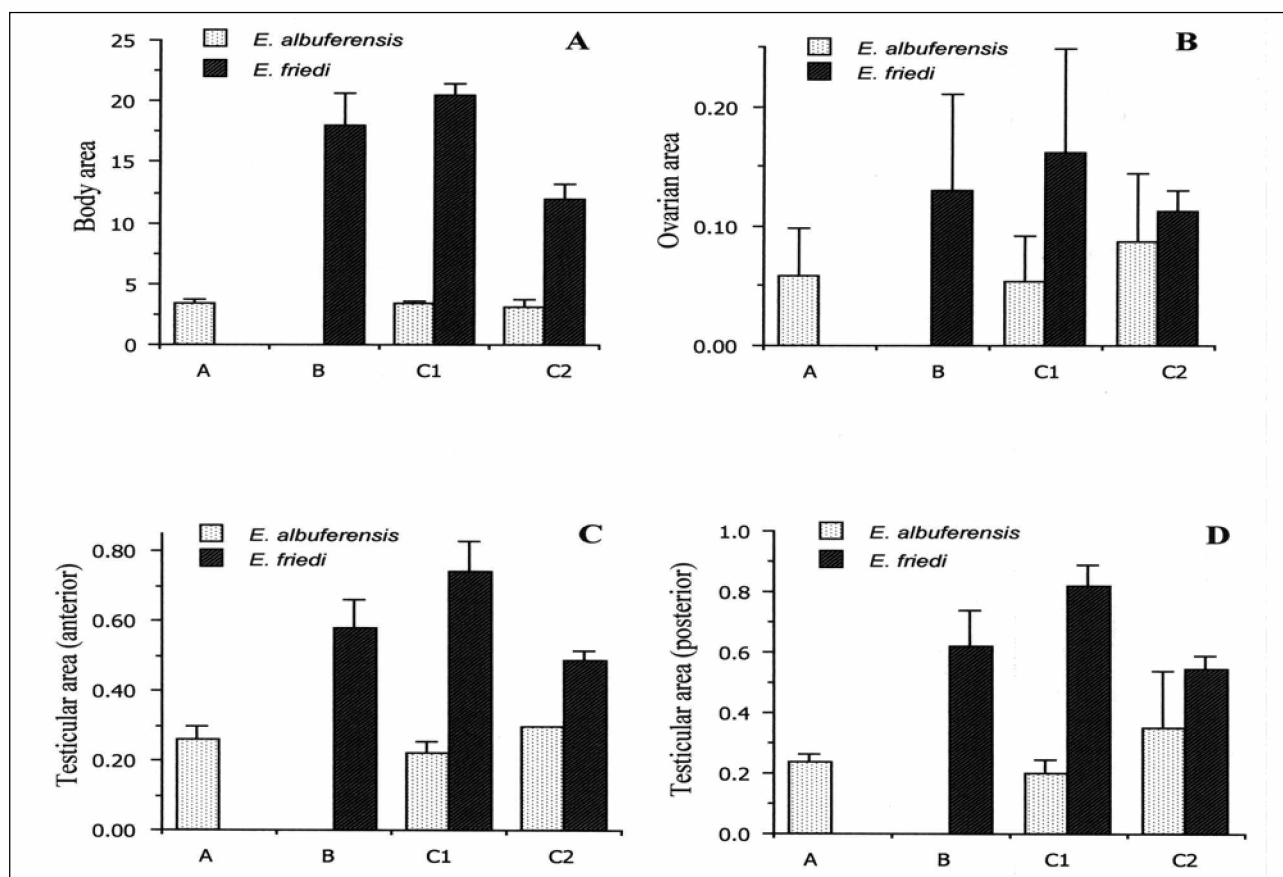


Fig. 1. Mean body area and gonadal areas (mm<sup>2</sup>) ± S.E. of *E. albuferensis* and *E. friedi* adult worms recovered in groups A, B – single infections and C – concurrent infections; C1 – worms recovered in different segments; C2 – worms recovered together in segment II. A – body area; B – ovarian area; C – anterior testicular area; D – posterior testicular area

without statistical differences.

In concurrent infections, distribution was more or less similar than in single infections for *E. albuferensis* worms, which were mainly located in segment I (88 %) with statistical differences ( $P (\chi^2 = 81.4) < 0.05$ ; D.F.: 136) towards worms in segment II (12 %). However, in the case of *E. friedi* most of the worms remain in segment II (60 %) with statistical differences ( $P (\chi^2 = 7.5) < 0.05$ ; D.F.: 170) with those placed in segment III (40 %).

There is no statistical difference in any of the intestinal segments between single and concurrent infections in any of the two parasite species considered.

Mean body area measurements (length x greatest width) from the three groups are shown in Fig. 1A. *E. friedi* body area was statistically greater than that of *E. albuferensis* in single and concurrent infections ( $P (t = 5.5 \text{ and } 19.67) < 0.05$ ; D.F.: 8 and 4). In concurrent infections when worms were separately recovered (*E. albuferensis* in segment I and *E. friedi* in segment III), although *E. albuferensis* slightly diminished its body area while *E. friedi* slightly increased, no significant differences occurred between single and concurrent infections in either of the two parasite species considered. However, when worms were recovered together (both parasite species in segment II), while *E. albuferensis* did not show significant differences in the body measurements compared with worms of single infections, *E. friedi* worms had significantly shorter body lengths not only than *E. friedi* worms of single infections ( $P (t = 3.85) < 0.05$ ; D.F.:10) but also than *E. friedi* worms of concurrent infections but recovered separately ( $P (t = 5.88) < 0.05$ ; D.F.:10).

Mean gonadal areas (ovarian and testicular) of the same worms used for the body area measurements are represented in Fig. 1B, 1C and 1D. Again, although *E. albuferensis* diminished its gonadal measurements, while *E. friedi* increased them, not significant differences occurred between single and concurrent infections in either of the two parasite species considered when worms were recovered separately. However, when worms were recovered together, while *E. albuferensis* did not show significant differences in the gonadal measurements compared with worms of single infections, *E. friedi* worms had significantly less anterior testicular length than *E. friedi* worms of single infections ( $P (t = 2.99) < 0.05$ ; D.F.: 9) and than *E. friedi* worms of concurrent infections but recovered separately ( $P (t = 5.98) < 0.05$ ; D.F.: 9).

## Discussion

*E. albuferensis* showed a 13 % recovery rates in single infections in hamster and 14 % in concurrent infections, while *E. friedi* increased from 8 to 17 % in concurrent infections. Such variability in infection rates was also observed in other echinostome species such as *Echinostoma paraensei* that showed an increase in infectivity from 15 to 31 % in concurrent infections in mice, while *Echinostoma caproni* recovery rates in concurrent infections (34 %)

were much less than in single infections (61 %) (Meece and Nollen, 1996).

*E. albuferensis* showed a marked specificity to its microhabitat in its definitive host, in that the first portion of the small intestine was the most suitable site for infection, whereas *E. friedi* was more adaptable to a wider range of intestinal sites than the former species, being able to extend beyond from the second third to the last intestinal portion.

Worm distribution was not affected for either species in the co-infection. Although the major infective sites within the host differ between the two species and a spatial separation of *E. albuferensis* (first third) and *E. friedi* (second third) was observed in the small intestine, in a concurrent infection these two parasite species can share the same microhabitat (second third) and can co-exist along the intestine of the definitive host without a clear interactive site segregation. However, Baruš *et al.* (1974) demonstrated how in a double infection with species belonging to different genera, *Echinostoma revolutum* and *Echinoparyphium recurvatum*, the final distribution of both fluke species in the intestine of the definitive host was the result of antagonistic interactions between both parasite species.

Several worm distribution patterns have been observed for different echinostome species coinfections. Iorio *et al.* (1991) found that worm distribution was not affected in a *E. caproni*-*E. trivolvis* coinfection in mice. However, Fried *et al.* (1997) reported the opposite situation for *E. revolutum* and *E. trivolvis*. In single infections *E. revolutum* was always found in the rectum, whereas *E. trivolvis* was at the lower ileum. In concurrent infections, both were found in the rectum. A change of worms location within the intestinal habitat can occur as Nollen (1997) observed in the case of concurrent infections with *E. caproni* and *E. trivolvis* in hamster.

Under conditions of crowding, a change in the normal habitat could be possible (Franco *et al.*, 1988; Yao *et al.*, 1991; Kaufman and Fried, 1994) as occur in multiple infections where the worms tended to locate more posteriorly than in single worm infections (Fried and Alenick, 1981). Moreover, a change in the normal distribution occurred at various times of infection. Meece and Nollen (1996) noted different locations from 14 to 21 d.p.i. in *E. caproni* and *E. paraensei* within simultaneously infected mice. In any case, in the present work all the necropsies were done the same d.p.i. and the effect of time of infection in worm distribution could not be investigated.

When *E. albuferensis* and *E. friedi* shared the same microhabitat in concurrent infections the antagonistic interactions selectively only affected to *E. friedi* worms that showed statistical diminution in its body and gonadal measurements. This antagonistic effects also occur in *E. caproni*-*E. trivolvis* coinfections, whose mean body areas were significantly decreased when compared to single species infections (Iorio *et al.*, 1991). However, these antagonistic interactions could be possibly due to the elevated *E. friedi* infective dose, in which factors released by worms asso-

ciated with crowding may be important on worms growth. Franco *et al.* (1988) showed how *E. revolutum* reduces its body size as a product of intraspecific competition.

In conclusion, in concurrent infections a balance seems to appear and although *E. friedi* adults seem to grow less, a greater worms recovery rate was obtained in the small intestine. The co-existence of intestinal helminths without high levels of interaction to avoid major competitions could be a fact of co-evolution in the same natural areas using the same definitive host. However, it is still not known what mechanisms and host cues govern the orientation and how echinostomes select their microhabitats in the intestine.

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