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INFLUENCE OF NON-HOST SNAILS ON THE DEVELOPMENT OF TISSUE LESIONS IN FIVE PULMONATE SPECIES AFTER THE PENETRATION OF *FASCIOLA HEPATICA* MIRACIDIUM

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ABSTRACT: Tissue lesions were studied in five aquatic pulmonate species infected individually by *Fasciola hepatica* or in pairs of mixed species (one miracidium per snail pair) on the second day after miracidial exposure. Juvenile and adult snails were used. No significant difference in survivals was noted between the different snail groups at day 2 of the experiment. However, significantly higher prevalence of necrosis was demonstrated i) in the albumen gland, the digestive gland, and the gonad versus the kidney, and ii) in snails infected in pairs of mixed species versus individually-infected controls and noninfected snails originating from pairs of mixed species. Age or species of snails have a restricted influence on prevalence of necrosis. Localized epithelial necrosis was the most frequently encountered lesion.

KEY WORDS: *Aplexa hypnorum, Bulinus truncatus, Fasciola hepatica*, histopathology, kidney, *Lymnaea truncatula*, miracidium, Mollusca, *Physa acuta, Planorbis leucostoma*, Trematoda.

INTRODUCTION

In a wildlife community, host molluscs living with other snail species ensure the development of trematode parthenitae. The influence of the presence of these non-host snails on the parasite cycle in host snails has been investigated by several workers. The miracidia can be lured by the presence of the non-host snails, thus reducing the infection success in the host snail (CHERNIN, 1968; CHRISTENSEN, NANSEN & FRANDSEN, 1976; LARACUENTE, BROWN & JOBIN, 1979; for example). Cercarial productivity through the host snail may be decreased (FRANDSEN & CHRISTENSEN, 1977) or significantly increased according to COMBES & MONE (1983) and MONE, THERON & COMBES (1986), the extent of the increase depending on the non-host snail species.

Few data are currently available on the pathology observed in snails during the sporocyst migration of *Fasciola hepatica*. A preliminary study reported the frequency of epithelial necrosis in three glands of seven pulmonate species on the second day after miracidial exposure. In the kidney, this necrosis was detected associated with intralamellar deposits, or edema (PREVERAUD-SINDOU & RONDELAUD, 1992). These initial findings raised the following questions. Has the presence of non-host snails an influence on the visceral pathology which develops in the host snail? Do these modifications of visceral pathology influence the survival of snails, in particular that of *Lymnaea truncatula*, the natural intermediate host of *F. hepatica*? To answer these questions we have studied the prevalence of tissue lesions in *L. truncatula*, and in non-host snail species such as *Aplexa hypnorum, Bulinus truncatus, Physa acuta*, and *Planorbis leucostoma.*

MATERIAL AND METHODS

Experimental protocol

Adults and newborns of the five snail species were used in this experiment. All of them originated from eggs that were laid by snails maintained in standard breeding containers. Adult height was from 4 to 5 mm depending on the species; newborn height was from 0,5 to 1 mm.

The design of the study was to place two snails, each of a different species, in contact with a single *F. hepatica* miracidium. A group of two hundred pairs was formed, each pair comprising an adult *L. truncatula*, and an adult of another species (*A. hypnorum, B. truncatus, P. acuta*, or *P. leucostoma*). Another group of two hundred pairs comprising newborns was formed according to the same protocol.

The two snails of each pair and the miracidium were placed in a 35-mm diameter petri dish, with 2 or 3 ml of water from a breeding container. Contact was maintained for 4 hours at 20° C. After exposure, the snail pair was placed in a 8-cm diameter Petri dish with 10 ml of water and pieces of lettuce for 24 hours. The snails were subsequently killed by immersing them in Bouin's fixative followed by immediate breaking of the shell. Serial sections (5 μm thick) were stained with Harris' hematoxylin and modified Gabe's trichrome.

Controls were established for each species by subjecting single snails to one exposure of a single miracidium (20 per species and snail age).

Characterization of lesions and statistical analysis

The lesions observed in the kidney consisted of i) necrosis of the lamellar epithelium, ii) intralamellar and/or perivisceral edema, and iii) intralamellar deposits. In the albumen gland, the digestive gland, or the gonad, there were i) localized epithelial necrosis of multifocal type, involving a limited area of the organ, and ii) generalized necrosis which extended throughout the gland. The characteristics of these lesions have already been described in previous reports (SINDOU, CABARET & RONDELAUD, 1991; PREVERAUD-SINDOU & RONDELAUD, 1992).

Categories		*Bulinus truncatus*	*Lymnaea truncatula*	*Physa acuta*	*Aplexa hypnorum*	*Planorbis leucostoma*
Adult	N	20	20	20	20	20
controls	S	20	20	20	20	20
	I[1]	19	20	18	20	20
Newborn	N	20	20	20	20	20
controls	S	19	20	20	18	17
	I[1]	18	20	19	18	17
Adult snails	N	50	200	50	50	50
in pairs of	S	49	200	50	50	48
mixed species	I[1]	24	113	21	20	20
(pms)	NI	25	87	29	30	28
Newborn snails	N	50	200	50	50	50
in pairs of	S	50	185	44	47	50
mixed species	I[1]	17	109	14	20	20
(pms)	Ni	33	76	30	27	30

Table 1.— Characteristics of controls and snails exposed to miracidia. Number of snails at the beginning of the experiment (N), number of survivors (S), the snails infected individually or in pairs (I), and those noninfected, kept in pairs (Ni). [1] = the infected snails were recognized by the presence of sporocysts and/or of tunnels of variable length which resulted from sporocyst migrations through the body of their host.

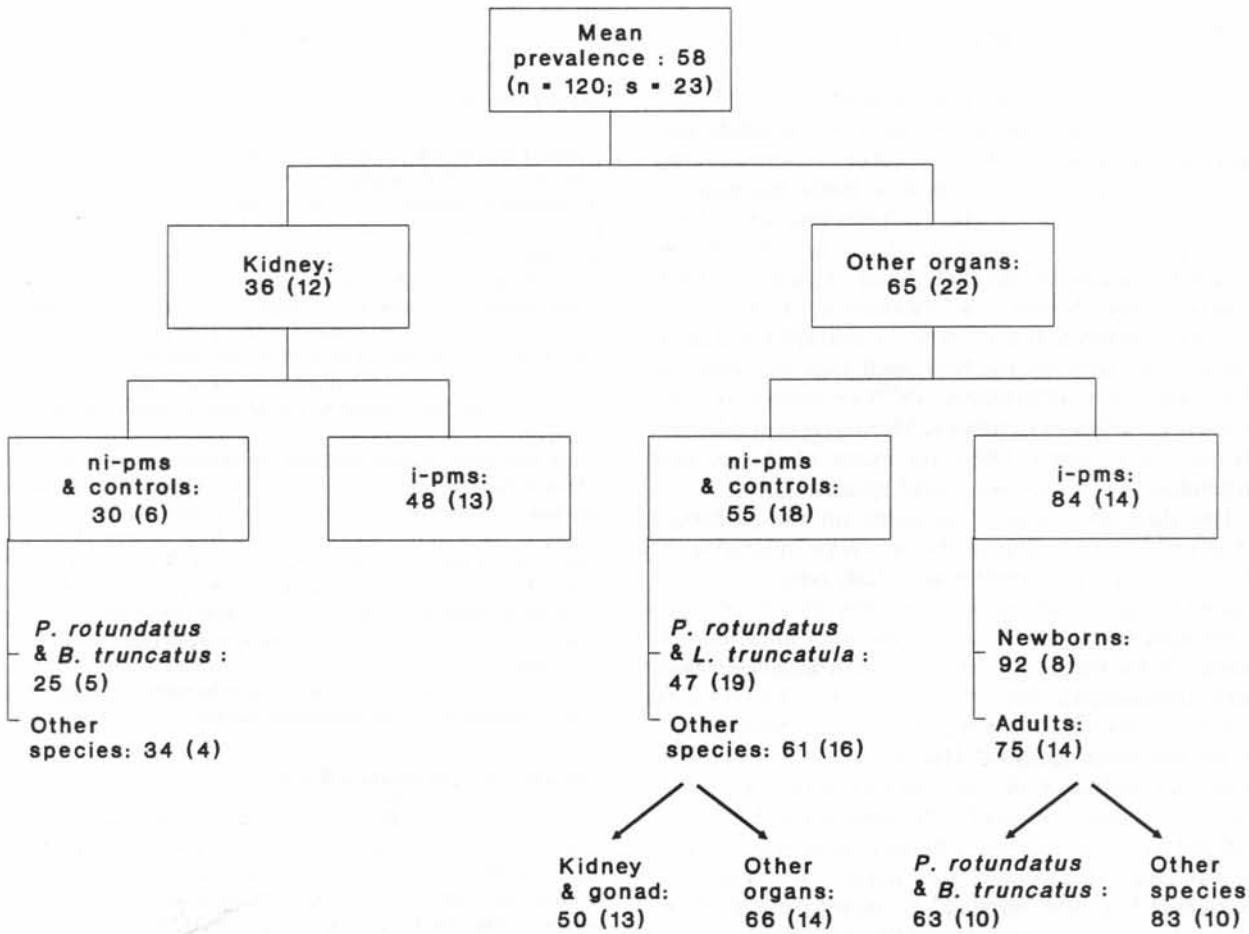

Fig. 1.— Localized and generalized necrosis: significant sub-groups of molluscs infected with *Fasciola hepatica*. i-pms = snails infected in pairs of mixed species; ni-pms = snails noninfected and kept in pairs of mixed species. Controls were individually infected snails. Numbers are prevalences of necrosis and those in parentheses correspond to their standard deviations.

Prevalences concerned the percentages of snails with a viscus affected by a particular lesion. Lesion prevalences were determined in relation to all possible structural appearances of each organ (or 100%).

Prevalences of necrosis (localized and generalized) were analyzed by means of segmentation analysis with a STAT-ITCF (1988) computer programme. The numerical dependent variable (prevalence of necrosis) was related with categorical independent variables (type of infection, species and categories of snails, and organs). The established sub-groups were the result of maximization of sums of squares of deviate for necrosis values (SSD) in relation to each independent variable. The SSDs were calculated as follows: SSD = n1 (%N1−%N) + n2 (%N2−%N), with %N prevalence of necrosis in the group to be subdivided, %N1 and %N2 were respectively mean prevalence of necrosis in the categories of independent variable (such as adult and juvenile snails), n1 and n2 were the number of snails

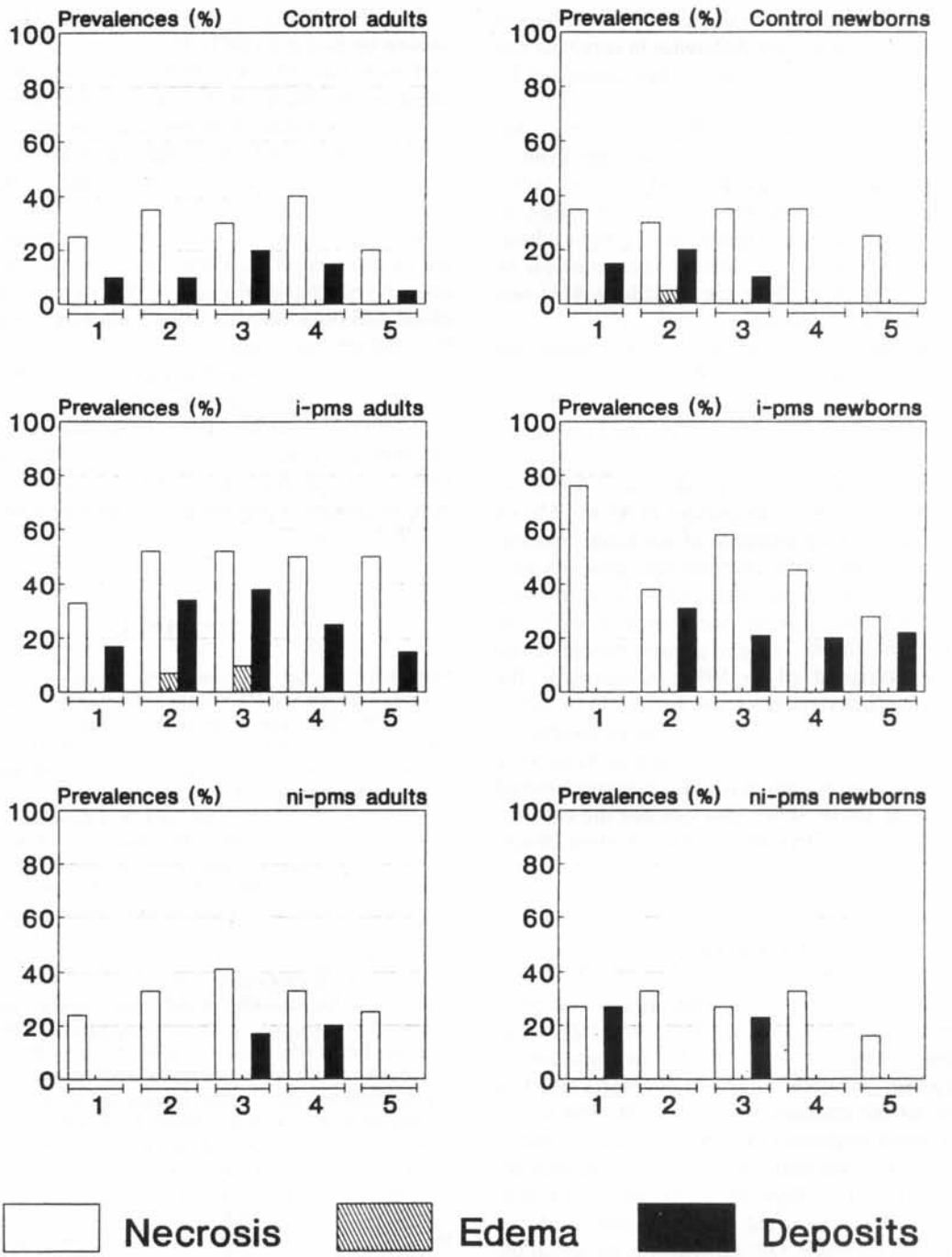

Fig. 2.— Prevalence of tissue lesions (No. of snails with lesion/No. of snails tested) in the kidney. 1 = *Bulinus truncatus*; 2 = *Lymnaea truncatula*; 3 = *Physa acuta*; 4 = *Aplexa hypnorum*; 5 = *Planorbis leucostoma*; i-pms = snails infected in pairs of mixed species; ni-pms = snails noninfected and kept in pairs of mixed species. Controls are individually infected snails.

in each category. A group was valid when the mean values of necrosis (%N1 and %N2 in the example) differed significantly (t-test; P = 0,95).

RESULTS

Table 1 indicates the number of surviving snails at day 2 of the experiment, those which were infected individually (controls) or in pairs of mixed species (i-pms snails), and those noninfected and kept in pairs of mixed species (ni-pms snails). No significant difference in survivals was noted between the different groups when compared by Chi2-test.

Prevalences of necrosis in relation to recorded parameters are in Fig. 1. The necrosis was significantly higher in other organs than in kidneys (65% versus 36%); type of organ was thus the most important factor in prevalence variations. In all organs, the i-pms molluscs showed higher prevalences of necrosis than controls or ni-pms snails (48% versus 30% for the kidney, 84% versus 55% for the other organs).

Age of snails had an influence only in i-pms group and in the other organs than kidneys (92% for newborns, 75% for adults). Species of snail had a restricted influence on prevalence of lesions; *P. leucostoma* presented the lowest necrosis prevalences.

Fig. 2 shows, for example, the prevalence of lesions in the kidney. Normal tissue was present in 45 to 75% of the control snails. The prevalence of epithelial necrosis ranged between 20 and 40%. Intralamellar deposits were occasionally absent; when they were present, they involved 5 to 20% of the snails. Edema was present in only 5% of newborn *L. truncatula*. In i-pms groups, normal tissue was likewise decreased (0 to 50%). Conversely, the prevalence of epithelial necrosis was higher (33 to 76%), as were deposits (15 to 38%). Edema was often absent. In ni-pms snails, the kidneys were normal in 41 to 84% of cases. Even though epithelial necrosis was encountered rather frequently (16 to 41%), this was not the case for the other two lesions. Deposits were most often absent and there was no edema.

DISCUSSION

Our results demonstrated that visceral lesions were more severe in pairs of mixed species than in controls. Since these findings were observed in the five species of pulmonate snails, it implies that a factor exists which is independent of the parasitic invasion itself. The factor in question could originate from the secretions or excretions of one or the two snails present. These substances would have a harmful effect on its partner and would worsen tissue lesions induced by the presence of the sporocyst in infected snails. Our experiments did not allow us to determine the extent of the role played by this factor and that related to the direct parasitic invasion of the infected snails. The results of MONE, THERON & COMBES (1986), however, adduce the question of the factor specificity because *Helisoma duryi* was the sole non-host species which had no influence on the cercarial productivity of *Schistosoma mansoni* by *Biomphalaria glabrata*. Further works should be carried out to determine the nature of this factor and the mechanism responsible of the origin of this worsening effect in the snail host.

The characteristics of the epithelial necrosis were, for the most part, identical with those reported by PAN (1965) in *B. glabrata* infected by *S. mansoni* or with the observations by BARBER (1962), RONDELAUD & BARTHE (1983) or ITAGAKI & ITAGAKI (1986) in several species of lymnaeid snails infected by *F. hepatica*. The development of generalized necrosis in certain snails examined on the second day might only be explained by an acute reaction to a toxic substance related to the presence of the sporocyst which soon lead to the snails' death. This hypothesis is based on histological observations of metaldehyde poisoning in slugs (SPARKS, 1985; TRIEBSKORN, 1989, 1991): a generalized epithelial necrosis detected in the digestive gland and the kidney of slugs a few hours following the first poison ingestion.

Edema and intralamellar deposits in kidneys were encountered with decreased prevalence in controls and in pairs of mixed species. Since the deposits are also found in snails in the absence of a parasitic infection (SINDOU, RONDELAUD & BARTHE, 1990), it is logical to presume that the process is physiological, although more developed in infected snails.

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