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NOTE

Palaemon elegans, an intermediate host in the life-cycle of *Aggregata octopiana*

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ABSTRACT: Based on immunological methods, the prawn *Palaemon elegans* apparently acts as the natural intermediate host for *Aggregata octopiana*, a coccidian that infects the cephalopod *Octopus vulgaris*. However, cross-reactivity with another coccidian, *A. eberthi*, was present in both sporogonic and merogonic stages.

KEY WORDS: *Aggregata octopiana* · Life cycle *Palaemon elegans*

Coccidians of the genus *Aggregata* cause major diseases in cephalopods. The merozoites migrate through the epithelium of the digestive tract causing degeneration and death of the parasitized cells and leading to detachment of necrotic fragments of the intestine (Hochberg 1990). Despite their importance, reliable information currently exists only about the life-cycle of the species *A. eberthi* Labbé, 1895, a coccidian parasite of crabs and cuttlefish. As in some other coccidians, there is alternation of generations and alternation of hosts. In both hosts, the parasites are found in the intestinal epithelium or wall. The asexual phase is in the peri-intestinal tissue of *Portunus depurator* L., 1758 while the sexual phase occurs within cells of the submucous connective tissue of the caecum and intestine of *Sepia officinalis* L. 1758 (Dobell 1925).

The cephalopod *Octopus vulgaris* Cuvier, 1789 is the definitive host for the species *Aggregata octopiana* Schneider, 1875 (Estévez et al. 1996). This paper presents the first report of the prawn *Palaemon elegans* Rathke, 1837 as intermediate host in the life-cycle of this coccidian.

Materials and methods. ***Prawns and parasites:*** Forty-eight crustaceans belonging to the species *Palaemon elegans* (Decapoda: Palaemonidae) (Haywar & Ryland 1985) were caught in the Ría de Vigo (NW Spain) and dissected. Their digestive tracts were extracted and examined under the stereo microscope in order to detect cysts. Oocysts of *Aggregata octopiana* and *A. eberthi* were isolated from the digestive tracts of the naturally infected molluscan cephalopods *Octopus vulgaris* and *Sepia officinalis*, respectively, in the Ría de Vigo (Estévez et al. 1996, Pascual et al. 1996).

Purification of sporocysts and preparation of sporocyst extracts: Sporocysts of *Aggregata octopiana* and *A. eberthi* were purified from oocysts. Briefly, sporocysts were obtained by maceration of infected, cephalopod host tissues in phosphate-buffered saline (PBS), pH 7.2. The resulting suspension was then filtered through increasingly fine meshes to remove tissue fragments. The filtrate was then centrifuged at $2000 \times g$ for 15 min. Filtration-centrifugation of the pellet was repeated several times until a pure sample of sporocysts was obtained. Sporocysts were lysed by sonication on ice (60 W in 1 min pulses for 45 min) and centrifuged at $10000 \times g$ for 30 min at 4°C. The supernatant (used in all analyses) was stored at –30°C until used (Leiro et al. 1993).

Collection of sera: Ten female Balb/c mice were immunized by subcutaneous injection of a 0.2 ml mixture of Freund's complete adjuvant (Sigma Chemical Co., St. Louis, MO, USA) and PBS containing about 5×10^6 sporocysts of *Aggregata octopiana*. They were boostered 30 d later by intraperitoneal (i.p.) injection of the same number of sporocysts in 0.2 ml of PBS without adjuvant. On Day 45 a third dose (without adjuvant) was administered by i.p. injection. Mice were bled 60 d after primary immunization through the retroorbital route. Serum (anti-*Aggregata octopiana*) was separated by centrifugation at $2000 \times g$ for 10 min, mixed 1:1 with glycerol and stored at –30°C until use. A similar protocol was used to obtain anti-*A. eberthi* serum.

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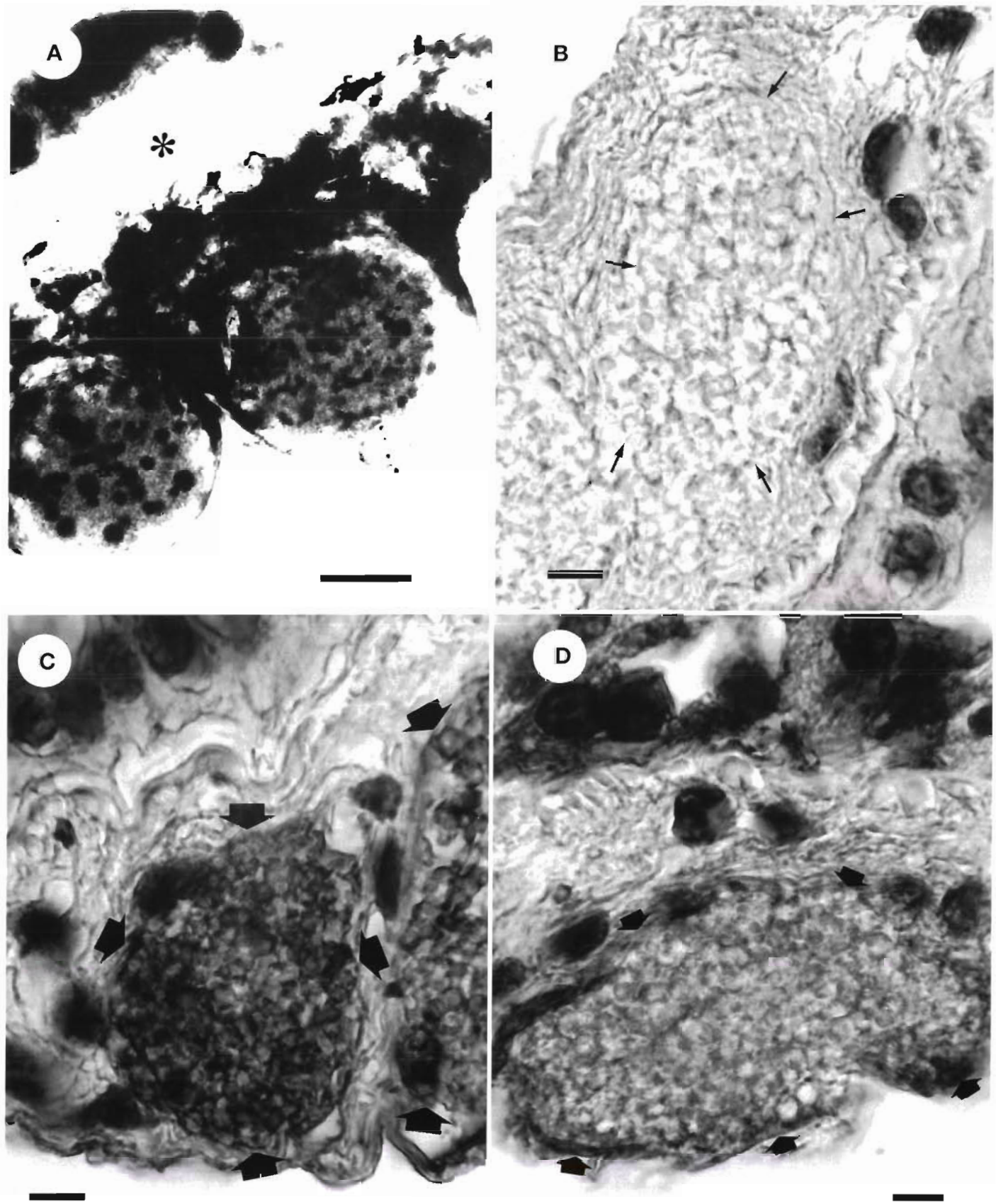
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Fig. 1. *Palaemon elegans*. (A) Segment of the gut of the prawn *Palaemon elegans* showing the intestine (*) and cysts with multiple nuclei arranged in the cytoplasm. Scale bar = 100 µm. (B–D) Section of a cyst in prawn gut tested with: (B) normal mouse serum, background level, (arrows indicate the cyst location); (C) anti-*Aggregata octopiana* mouse serum (arrows indicated the area of strong positive reaction), and (D) anti-*A. eberthi* mouse serum (arrows indicate the area of slight reaction). Haematoxylin was used as the counterstain. Scale bar = 10 µm

Slide pre-processing: Infected intestines from the crustacean *Palaemon elegans* were kept in 10% formaldehyde in PBS, pH 7.2, for 12 to 24 h. The samples were dehydrated in increasing concentrations of ethanol and embedded in paraffin wax, after which 4 µm microtome sections were cut, dewaxed and mounted.

Immunohistochemistry: Immunohistochemistry was performed by the indirect immunoperoxidase method (Tijsen 1987). Mounted sections were incubated for 30 min in 10% H_2O_2 in Tris-buffered saline (TBS: 50 mM Tris, 0.15 M NaCl, pH 7.4) to inhibit endogenous peroxidase. They were then washed with TBS and incubated for 1 h with 5% non-fat dry calf milk in TBS containing 0.2% (v/v) Tween-20 (TBS-Tween) at room temperature (RT) to block non-specific reactions. After being washed with TBS-Tween, the slides were incubated with a 1:20 dilution of the primary sera (anti-*Aggregata octopiana* or anti-*A. eberthi*) in TBS-Tween for 4 h at RT in a humid chamber. Next, they were washed with TBS-Tween and incubated for 1 h with polyclonal rabbit anti-mouse immunoglobulin serum (Dakopats A/S, Glostrup, Denmark) diluted 1:100 in TBS-Tween. The slides were then washed with TBS and incubated with 100 µl substrate solution of TBS containing 0.003% H_2O_2, 0.06% diaminobenzidine tetrahydrochloride (Sigma) and 0.03% $NiCl_2$. After a brown colour had developed, the reaction was stopped by washing the slides with TBS. The sections were then mounted in TBS-glycerol (1:1) and covered with a cover glass for photography. Appropiate negative controls were included in each case. Haematoxylin was used as the counterstain (Culling et al. 1985).

Enzyme-linked immunosorbent assay (ELISA): Titration curves were derived and the cross-reactivity of the antisera assayed was tested using the indirect ELISA technique, as described previously (Estévez et al. 1994).

Results. ***Immunohistochemistry:*** At least 1 cyst was found to be present in the peri-intestinal tissues of 63% of the natural *Palaemon elegans* examined. Fig. 1A shows these cysts, inside of which multiple nuclei can be seen, corresponding to the merozoites.

Intestinal sections of *Palaemon elegans* containing cysts were chosen for immunoassay comparisons. Negative controls used normal mouse serum (background level) which gave no reaction (Fig. 1B). In all the coccidian-infected prawns tested, the cysts containing merozoites reacted positively to anti-*Aggregata octopiana* sporocyst mouse serum (Fig. 1C) but showed little reaction to anti-*A. eberthi* sporocyst mouse serum (Fig. 1D).

Cross-reactivity study: ELISA was used to determine the titer of the 2 polyclonal antisera, which was estimated at over $1:10^6$ for both (Fig. 2A). Cross-reactivity was also observed. Antibodies raised in mice with *Aggregata eberthi* purified sporocysts cross-reacted with sporocyst extracts of *A. octopiana* (heterologous antigen) (Fig. 2B).

Discussion. Polyclonal antisera are effectively applied in taxonomic studies on protozoa (McGovern & Burreson 1989, Martín-González et al. 1991) and here we used them to identify the intermediate host in the cycle of *Aggregata octopiana*.

Immunoassay of the prawn tissues indicated that mouse anti-*Aggregata octopiana* sporocyst serum gave a strong positive reaction to cysts (merozoites) found in

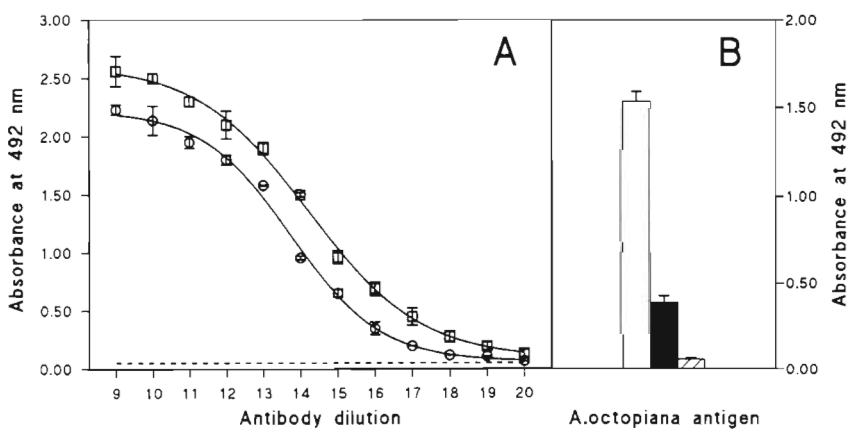

Fig. 2. (A) Titration curves for ELISA assays of sporocyst antisera. (□) Anti-*Aggregata octopiana* sporocyst mouse serum; (○) anti-*A. eberthi* sporocyst mouse serum. Background level (normal mouse serum) is represented by the dotted line. Values are expressed as $\log_2$ of the antibody dilution with initial dilution 1:500. (B) Cross-reactivity of the antisera assayed (at dilution 1:20000) with antigen extracted from *A. octopiana* sporocysts. White bar: anti-*A. octopiana* sporocyst serum; black bar: anti-*A. eberthi* serum; hatched bar: normal mouse serum

the digestive tract of *Palaemon elegans*. Nevertheless, a slight reaction was also observed between these cysts and mouse anti-*A. eberthi* sporocyst serum. Cross-reactivity between species of the same genus has already been recorded in coccidians of the genus *Eimeria* by Xie et al. (1992). Here, the cross-reactivity between the 2 species of *Aggregata* was shown to occur for 2 stages of the biological cycle (i.e. the merogony stage in the crustacean gut and the sporogony stage in the cephalopod gut).

The prevalence of cysts of *Aggregata octopiana* in the gut of *Palaemon elegans* was approximately 63%, a figure which comes close to that of 75% obtained by Dobell (1925) for *A. eberthi* in the crab *Portunus depurator*. However, Dobell showed that parasitic specificity of *A. eberthi* was slight, since at least 2 species of crab belonging to the genus *Portunus* (mainly *P. depurator* and to a lesser extent *P. corrugatus* Pennant, 1777) could be intermediate hosts. We do not rule out the possibility that other prawn species of the genus *Palaemon* could also act as intermediate hosts for *A. octopiana*.

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