Observations on associated histopathology with *Aggregata octopiana* infection (Protista: Apicomplexa) in *Octopus vulgaris*

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ABSTRACT: Gamogony and sporogony of *Aggregata octopiana* were commonly observed during histological examination of the digestive tract of wild *Octopus vulgaris* from Ría de Vigo (NW Spain). *A. octopiana* infected noncuticularized caecum and intestine, and cuticularized oesophagus and crop. Infection was also observed in the gills and in covering mesenterium, mainly of the digestive gland and gonad. Histological and ultrastructural lesions associated with *A. octopiana* included host cell hypertrophy with nuclear displacement, inflammation, phagocytosis, ulceration and destruction of organ architecture. The possible existence of a malabsorption syndrome in the host is deduced.

KEY WORDS: Apicomplexa · *Aggregata octopiana* · O*ctopus vulgaris* · Digestive tract · Histopathology · Defense mechanisms

INTRODUCTION

Coastal octopuses have the longest history of human exploitation of any cephalopod (Boyle 1990). The best known species, the common octopus *Octopus vulgaris* Cuvier, 1798, is extremely important in fisheries (Roper et al. 1984, Balguerías et al. 2000, Domain et al. 2000, Josupeit 2000, Pereira [www.fao.org/docrep/X3900e/X3900e9.htm]), and also has great potential as an aquaculture species. It also represents a major protein resource in most Mediterranean countries (Guerra 1992, Quetglas et al. 1998) and is widely used as a bio-medical research model (Hanlon & Forsythe 1985).

Despite their importance, little information is available on protozoan-related diseases occurring in wild octopus populations. A notable exception is the coccidian *Aggregata* Frenzel, 1885 (Apicomplexa: Aggregatidae) that has been reported in the digestive tract of 7 octopus species world-wide (Gestal 2000).

Coccidians within the genus *Aggregata* are intra-cellular parasites with a 2-host life-history that are transmitted through the food-web. Sexual stages occur in the digestive tract of cephalopod, the definitive host; asexual stages infect the digestive tract of crustaceans, the intermediate hosts (Hochberg 1990).

The presence and taxonomic identification of the genus *Aggregata* in cephalopods have been recorded by different authors (Moroff 1908, Dobell 1925, Narasimhamurti 1979, Sardella & Re 1988, Poynton et al. 1992, Estévez et al. 1996, Gestal et al. 1999). Observations on associated pathology of *Aggregata* infections in cephalopods is limited to *A. dobelli* and *A. millerorum* parasites of *Octopus dofleini* and *O. bimaculoides* respectively (Poynton et al. 1992).

Histological and ultrastructural lesions associated with *Aggregata octopiana* (Schneider 1875) in the digestive tract of *Octopus vulgaris* from the NE Atlantic coast are described herein.

MATERIALS AND METHODS

A total of 100 post-recruit common octopuses *Octopus vulgaris* was collected by traps in the Ría de Vigo, Galicia, Spain (NE Atlantic Ocean: 42° 15′ N, 8° 48′ W). At the laboratory, fresh individuals in post-mortem

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condition were necropsied. To confirm the presence of *Aggregata octopiana* oocysts, squash preparations of fresh material from all organs of the paleal cavity were analysed by light microscopy (LM). Infected tissue samples from target organs (caecum and intestine) were then fixed in 10% buffered formalin, dehydrated in a graded ethanol series, embedded in paraffin and sectioned using a Microm HM-340 E microtome (Microm). Sections (4 to 5 µm) were stained with hematoxylin-eosin following standard procedures (Culling et al. 1985).

Ultrastructural aspects of infected tissues were assessed by transmission electron microscopy (TEM). Small pieces of infected tissues were collected and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 4 h at 4°C, washed for 12 h at 4°C in the same buffer, and post-fixed in buffered 2% OsO_4 for 3 h at the same temperature. After dehydration in a graded ethanol series, the tissue was embedded in Spurr resin. Semi-thin sections obtained by diamond knife were stained with methylene blue. Ultrathin sections were double stained with uranyl acetate and lead citrate, and observed using a JEOL 100CXII TEM operated at 60 kV.

RESULTS

Gross pathology. White cysts containing *Aggregata octopiana* sporocysts were commonly found by gross observation in the digestive tract.

Site of infection. As seen by LM, the noncuticularized spiral caecum and intestine were the most infected organs, although infection also extended to the cuticularized crop and oesophagus (including the region adjacent to the radula). In massive infections, gill tissue and the covering mesenterium, mainly of the digestive gland and gonad, were also infected. Fig. 1 shows the relative percentage of the infected organs.

Prevalence. Of the octopuses examined, 98% (98/100) were infected at least in the caecum tissue.

Histopathology. In the target organs (caecum and intestine), parasites were most frequently found in the submucosa and muscularis, less commonly in the serosa.

Parasite development in the octopus includes gamogony, and sporogony. After fertilisation, formation of the zygote occurs, and a gross cyst wall develops. Invaded host cells undergo
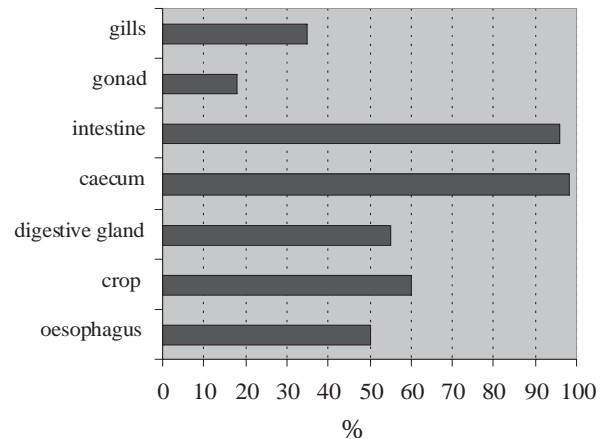

Fig. 1. *Octopus vulgaris*. Relative percentage of organs infected with *Aggregata octopiana*

considerable hypertrophy, and the nucleus appears to be displaced to one side (Fig. 2). Infection starts in the mucosal folds and spreads to the proximal submucosa and serosa layers. Frequently, marked distension of the infected tissue area due to the development of gametes or sporogonic stages is seen, causing rupture of the basal membrane and the detachment of the epithelial cells (Fig. 3A,B,C). Due to severe loss of epithelial cells, atrophy of mucosal folds occurrs in affected intestine and caecum. The mucosa of the caecum and intestine is ulcerated. Inflammation, pericyst reactions of connective tissue and infiltration of hemocytes in the area of ulcerated mucosa is observed (Fig. 3D,E,F). Fibrosis and necrosis are also occasionally observed. Finally, infected mucosal cells undergo
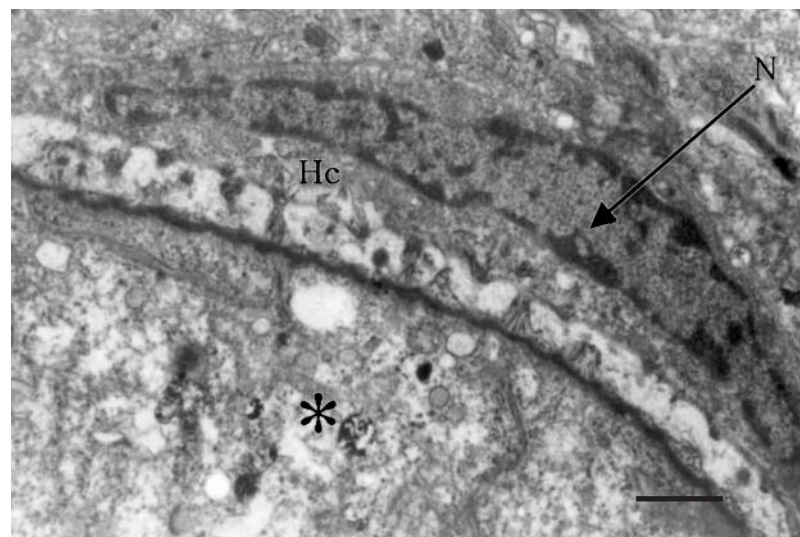

Fig. 2. *Octopus vulgaris.* TEM micrograph of a hypertrophied host cell (Hc) of the caecum infected by *Aggregata octopiana* (✱). Arrow shows the cellular nucleus (N) displaced to one side of the cell. Scale bar = 1.30 µm

necrosis. Different stages of gamogony and sporogony are observed in the caecum and intestine. Individual sporocysts not enclosed in cysts are present in the tissue. Sporogonic stages are phagocytosed by hemocytes (Fig. 4A,B). In heavy infections, most infected host tissue is replaced by parasites. Loss of intestinal epithelium and destruction of the tissue organ architecture is observed (Fig. 4C,D).

DISCUSSION

Although an extensive literature dealing with diseases and defense mechanisms is available for other commercially important molluscs, little emphasis has been placed on parasite-caused diseases, pathological effects of parasites, and defense-associated responses of cephalopods against specific pathogens (Hochberg 1990, Ford 1992).

Molluscs have both cellular and humoral mechanisms of defense (Ford 1992). The most widely reported defense mechanism in cephalopods is the cellular one (Malham 1996, Malham et al. 1997, Malham & Runham 1998). Hemocytes are important in phagocytosis and killing of infectious agents, and in the repair of tissue damage resulting from mechanical or biological trauma. Encapsulation and phagocytosis of foreign materials are primary roles of molluscan hemocytes (Ford 1992). Phagocytosis of foreign material has been described in coccidian infections in different marine host species (Lom & Dyková 1992) and birds (Hammond 1973).

The degree of damage caused by coccidia to their hosts depends upon the numbers of parasites occurring at any particular site. Thus, the degree of damage to the host caused by coccidia might be considered directly proportional to the degree of destruction of host cells. Long (1973) showed that there is a clear relation between the pathogenicity of coccidian species and the depth to which they penetrate the intestinal mucosa of the host. Hammond (1973) described the
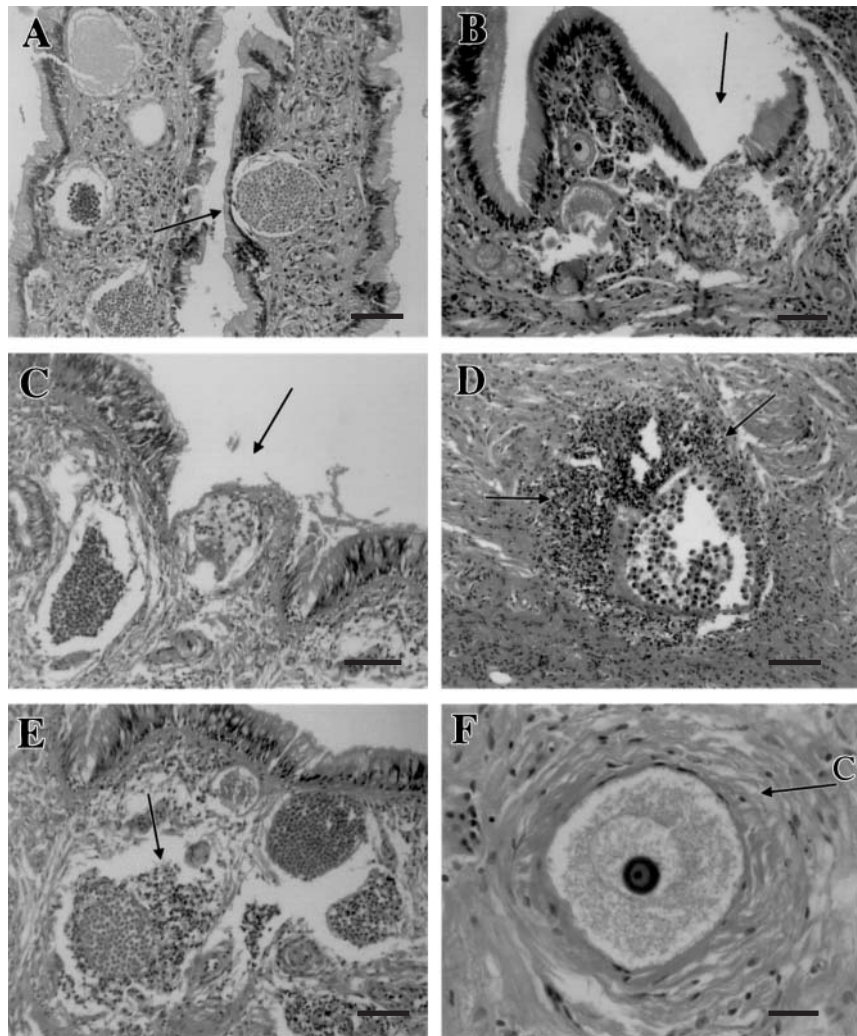

Fig. 3. *Octopus vulgaris*. Histological sections of the caecum and intestine infected with *Aggregata octopiana*. (A) Caecum villus infected with *A. octopiana* showing distension (arrow). Scale bar = 100 µm. (B, C) Distension and rupture of infected tissue from development of the coccidia; arrows show rupture of the basal membrane and loss of epithelial cells. Scale bars = (B) 100 µm, (C) 60 µm. (D), (E) Inflammatory reaction in response to the *A. octopiana* infection; arrows show strong infiltration of hemocytes surrounding *A. octopiana*. Scale bar = 100 µm. (F) Pericyst reaction (C) of connective tissue surrounding the parasite. Scale bar = 40 µm

pathologic effects of the coccidia genus *Eimeria*, showing a strong inflammatory infiltration and deformation of the intestinal villus of the host caused by the infection.

The first signs of pathology in the cephalopod intestinal infections by the genus *Aggregata* occur at the point of entry and during migration of the parasite through epithelial cells. This is due to the break of the junctions between cells, causing necrosis (Dobell 1925, Porchet-Henneré et al. 1981, Hochberg 1990). Dobell (1925) described by LM the destructive effects of *A. eberthi* infections in *Sepia officinalis*, and host cellular defense reactions against infection. This author indi-
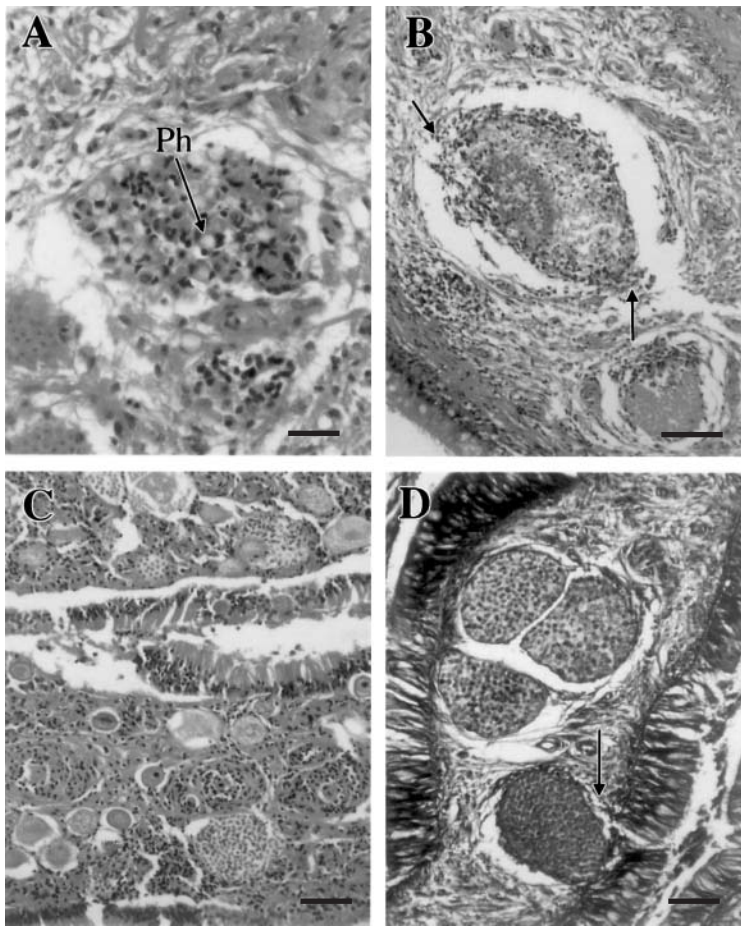
Fig. 4. *Octopus vulgaris.* Histological sections of the caecum and intestine infected with *Aggregata octopiana*. (A,B) Infected intestinal tissue showing *A. octopiana* sporocysts phagocytosed by hemocytes (Ph) (arrows). Scale bars = (A) 40 µm, (B) 60 µm. (C,D) Infected caecum showing distension (arrow in D) and destruction of the tissue organ architecture; the majority of heavily infected caecum area is replaced by parasites. Scale bar = 100 µm

trast, Poynton et al. (1992) indicated that gamogony and sporogony of *A. millerorum* occurred in the noncuticularized spiral caecum and intestine, and in the cuticularized oesophagus and crop of cephalopod hosts. However, another species, *A. dobelli* was exclusively present in noncuticularized portions of the digestive tract of its cephalopod host. The presence of *A. millerorum* in cuticularized portions of the digestive tract was interpreted by Poynton et al. (1992) as an increase of the invasive potential of this *Aggregata* species. Our results are not in complete agreement with the interpretation of Poynton et al. (1992). Although *A. octopiana* also has strong invasive potential, a high relationship between invasiveness and infection intensity should also be noted. Thus, heavily infected octopuses had a higher number of different infected organs (noncuticularized and cuticularized organs) than less infected octopuses (noncuticularized organs only).

Pathology caused by different *Aggregata* species in their cephalopod hosts is highly variable. These effects probably depend not only on the cephalopod host but also on the *Aggregata* species. Although we did observe signs of host defense, the host response of *Octopus vulgaris* to the *A. octopiana* infection appears limited. A limited defense response was also described by Poynton et al. (1992) for *A. dobelli* and *A. millerorum* in octopuses from Pacific waters.

Although infection by *Aggregata octopiana* is not believed to be the primary cause of host death in the present study, severe *Aggregata octopiana* infections may have weakened the octopuses, making them more vulnerable to other biotic and abiotic effects.

Even though gross clinical signs of disease were not evident in infected live octopuses (Gestal et al. unpubl. data), a high degree of invasiveness and pathogenicity by *Aggregata octopiana* may have detrimental effects on gastrointestinal function. A malabsorption syndrome is probably produced in the host through the destruction of the gut architecture. Therefore, the use of biochemical analyses to determine the effect of coccidiosis on the degree of activity of absorption enzymes and physiological characteristics including the condition, growth rate, nutrient assimilation and protein/energy ration of cephalopod hosts would be useful.

cated that in heavily infected cuttlefish the destruction of the gut mucosal membrane occurs, and most necrotic epithelium is shed into its lumen. Sheets of necrotic intestinal epithelium and mucous membrane leave the host with the faeces (Dobell 1925). In the present study, the use of wild individuals in post-mortem condition did not allow observation of any parasitized necrotic intestinal sheets leaving the host with the faeces. However, the histopathological results herein reported suggests that the effect of *A. octopiana* on *Octopus vulgaris* was similar or even greater than that described by Dobell in *S. officinalis.*

Parasitic forms of the genus *Aggregata* had previously been described exclusively in the epithelial cells of the mucosa and submucosa tissue of the intestine and caecum of infected cephalopods (Dobell 1925, Porchet-Henneré et al. 1981, Hochberg 1990). In con-

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